

Bigger sums for better sums

The Reagan Administration is expressing concern at declining standards of mathematics and science in schools. It needs to put its money where its mouth is.

A HOCKEY team has won 5 out of 20 games it has played: what percentage of games has it won? You have a master's degree in chemistry and head the science department of a Florida secondary school where you have taught for seven years. What is your salary?

The first question could not be answered correctly by 48 per cent of the American 17-year-olds to whom it was recently posed by the National Assessment of Educational Progress. The answer to the second question is \$18,000. Could there be a connection between the answer to question two and the inability of so many young Americans to find the correct answer to question one? On present showing, the Reagan Administration appears not to think so. Forced by public opinion to acknowledge that secondary education is facing a deep crisis, President Reagan has this summer adopted the cause of education as if it were his own. His pre-election pledge to abolish the Department of Education has been tactfully forgotten. In school visits around the country he has been televised reading from Shakespeare and advocating a return to the basics, more homework and merit pay for teachers. What he has refused to do is accept the simple proposition that the best way to improve the quality of the schools is to pay for better teachers.

There is now overwhelming evidence that it is the poor pay and status of teachers that has driven able new entrants from the profession. In science and mathematics the problem is especially acute. New teachers are being drawn from the bottom quarter of high-school graduates. From 1971 to 1980, the number of students training to teach dropped threefold in science and fourfold in mathematics, and only half of this group has actually taken a job in teaching. One in four of those who have say they intend to give up in the near future. The result: a massive shortage that is likely to become worse. In 1981, half the teachers newly employed to teach mathematics and science at secondary level were unqualified to do so. And the shortage will be exacerbated by attempts to raise the standards of teachers or of the children they instruct. Increasing the number of hours devoted to mathematics and science will push up demand while the insistence that new teachers pass minimal competency tests will push down supply.

In responding to the cacophony of recent reports that have made education into the kind of political issue it has not been since Sputnik, President Reagan has done his level best to avoid the obvious conclusion that teachers must be paid more if their calibre is to be improved. Part of his argument is constitutional: it is the states and school districts, not the federal government, that have the prime responsibility for paying teachers. The other part is merely rhetorical: federal spending on education has increased for 20 years without an obvious improvement in educational standards. Why should spending more on teachers be expected to provide a cure now?

The constitutional argument can be easily disposed of. Few states possess a tax base robust enough to pay for the kind of pay increases that would be needed to change the attractiveness of a career in teaching. There are, however, scores of precedents for federal help in the form of matching grants to enable states to achieve goals that serve the national interest and it is hardly possible to deny that the decline of secondary education standards, particularly in science and mathematics, is a national issue of the first order. The scientific ability of 17-year-olds,

measured by major national surveys in 1969, 1973 and 1977, has seriously declined. Scores on the Scholastic Aptitude Test (SAT) fell for 18 years until they levelled out two years ago. Between 1975 and 1980 the amount of remedial mathematics teaching offered by universities had to increase by more than 70 per cent while overall enrolments increased by only 7 per cent.

That pay can play a big part in the improvement of the quality of teachers has been partially acknowledged by the administration in its endorsement of the concept of "master teachers" — an elite cadre of teachers who would be paid above the standard rate and serve as an example and incentive to newcomers to the profession. Several states — notably California and Tennessee — appear to be moving rapidly towards the adoption of such schemes. Under pressure from their members, the teachers' unions have begun to soften their opposition to master-teacher plans, while insisting that the longer-term objective should be to raise the general salaries of all teachers. Here too, however, the administration has refused to put its money behind its professed convictions. If there are to be enough master teachers receiving big enough salaries to change the image of teaching, federal help is essential, particularly in states such as Michigan where local economies are incapable of sustaining increased expenditure.

Paying teachers more is not a guarantee that standards of education will improve. But continuing to pay them too little is a guarantee that standards will continue to decline. Why is it that between the ages of 9 and 17 there is a huge reduction in the interest children take in science at school, while they continue to be fascinated by science in the newspapers, in science parks and on television? One reason is that a proper understanding of science — as opposed to a superficial savouring of its delights — requires some disciplined work. The other is that teachers are paid less than television producers. □

Money for AIDS

British spending too little, US research will need control

THERE is no better measure of the extent of the publicity that has attended the dramatic rise of AIDS than the fact that less than two years after its recognition and with only about 2,000 cases worldwide, the acronym for acquired immune deficiency syndrome is a household term. Calculations that the number of cases of AIDS doubles every month and its alarming mortality rate have led to an understandable degree of apprehension, even panic, in groups at risk. Has the response from the biomedical community and its sponsors been adequate?

Shortly after it first became clear how serious was the AIDS problem, there were cries from the homosexual community, some plaintive, some accusatory, that the problem was being ignored because it affected a minority group. Moreover, there was a feeling that criticism of the promiscuous lifestyle of some networks of homosexuals, a large risk factor in AIDS, was more in evidence than was compassion. These early reactions were misplaced. Compassion is not lacking from the medical or the biomedical professions; money for new lines of research does not grow on



trees; and there is always a lag, born of caution and commitments, before investigators switch their interests. In any case, even without money or compassion, scientists would have turned to research on AIDS. The challenge of finding the cause or devising effective treatment and the kudos to be gained from success with either would have been irresistible.

With the interests of the biomedics aroused, the next question became would they have projects worth supporting and would there be sufficient funds to meet them? In the event, especially since it was an American event, the second question has tended to eclipse the first. At the latest count (see page 677), the National Institutes of Health are likely soon to have a total of \$48 million to pour into AIDS research, \$24,000 for each of the 2,000 cases of AIDS so far recorded. The National Institutes are speeding up their usual review process for grants and awarding relatively huge sums of money to some investigators.

Will the money be well spent or are all the haste and dollars a recipe for much poor research, an easy ride on the bandwagon? There is a temptation to compare these events with the great drive against cancer initiated by President Richard Nixon in the early 1970s. As some predicted and many concluded, the huge sums of money made available through the National Cancer Institute bought a lot of poor research and, on the whole, poor returns. It would, however, be a mistake to draw too direct a comparison between the two because an immediate focus on AIDS is justified in a way that it was not on cancer. The reason is that AIDS is a new syndrome, spreading rapidly, where the early phases of the spread can and should be vigorously investigated.

Already, the initial phase may have passed in the United States. In the United Kingdom, the situation may be different, with only sixteen recorded cases of AIDS but suggestive evidence of many more cases in a latent phase of the disease. An intensive effort now could therefore contribute much to an understanding of the initial spread of AIDS through a population.

The UK Medical Research Council has so far shown little enthusiasm for the task. It has awarded just one grant of £40,000 for work on AIDS, although other applications are being considered and a special advisory committee on AIDS is soon to be convened. The council has no plans to earmark any money for AIDS research and will treat applications on their scientific merits. In other circumstances, that cool and conservative approach would be commendable, but the problem of AIDS and the opportunity for an intensive study of its early phase call for flexibility on the part of the Medical Research Council. It should at least designate AIDS research as an area of priority, if only to encourage applications. Moreover, it should process applications with speed and be prepared to be less rigorous than usual about the quality of proposals in recognition of the need for urgent studies. In so doing, it will have to risk wasting money on relatively poor or unnecessary projects. But the risk is worth taking, particularly since the Medical Research Council should easily be able to turn off the tap when the time comes. The National Institutes of Health, which might consider supporting UK research on AIDS whether or not national funds are forthcoming, needs to plan now how it will control and eventually curtail the programme of AIDS research it has started. □

As stubborn as a mule

US agricultural research would benefit greatly from more competitive grants.

THE days of fistfights on the floor of the US House of Representatives are long gone; these days, the real blood is spilled behind the scenes. Representative Jamie Whitten of Mississippi, a member of Congress for more than 40 years and chairman of the all-powerful Appropriations Committee, is a skilled practitioner of the quiet blow, especially when it comes to reform of agricultural research policy. This year, once again, Mr Whitten has succeeded in killing off even the most modest proposals for reform of America's archaic and byzantine agricultural research system.

What Mr Whitten has done this time is to delete a modest \$4 million increase that the administration had proposed for the small competitive grants programme in agriculture. This programme, established five years ago as an alternative to the traditional research structure, was supposed to bring to bear on agriculture the expertise traditionally excluded and, in theory, available at non-land grant universities were they to bring their intellectual capital and the tools of molecular biology to bear upon plant genetics. Mr Whitten, however, has never allowed the funding level of the competitive grants to rise above what can only be called a token level: \$17 million next year. This is in sharp contrast to the nearly \$250 million that the federal government will dole out to the land-grant colleges and their associated state experiment stations according to a hundred-year-old formula that remains unfettered by such distractions as having to judge the scientific merit of the research it supports.

Mr Whitten's rationale for this position is instructive, not least for what it says about how far agricultural research in the United States has grown from the kind of science that should now be underpinning it. The danger of competitive grants, he says, is that Congress cannot "ride herd" over them. Left to their own devices, researchers go off and study things that they just happen to get interested in — why, Mr Whitten says, he discovered that some researchers were still studying mules years after tractors had replaced the last of them. And what's more, Harvard and other such institutions may not really be able to do that much for agriculture — the real business of agricultural research is testing new varieties; for instance, adapting soybeans to the soils and hot climate of the South.

Mr Whitten's conception of agricultural research might simply be considered quaint if it were not pernicious. For his devotion to the land-grant system's nineteenth-century views of agriculture and science is unfortunately squarely in conflict with the urgent need to replenish the scientific capital that applied research must draw on if progress is to be made. The facts of recent years leave little doubt about the urgency of this need and the vastness of its potential. Yields of many crops are levelling off after years of gains; costs of production continue to climb; and the triumphs of traditional applied research and traditional plant breeding are facing the point of diminishing returns. Sprinkling on a little more fertilizer is no longer the recipe for success.

Yet efforts applied to serious research on plant molecular biology remain feeble. The few exceptions within the land-grant system, such as Cornell University and the University of California at Davis, only illuminate the problem: their existence owes as much to their research reputations as to their state and federal financial support through the land-grant formula funds. The need for local applied research of the sort the land-grants have encouraged so well will, of course, always be necessary. Unfortunately, that indisputable need has become the rallying point for those infatuated with the *status quo*. "Don't fix it if it ain't broke" has become the rallying cry of the 40 or so experiment station directors who surely must know that their research on square tomatoes, plastic roughage for dairy cattle or new uses for soy meal would never rank at the top of a competition for funds based on scientific merit. Basic research and competitive grants are to them, sad to say, threats rather than opportunities.

Unless the agricultural establishment quickly drops its self-assured equation of basic research with studying mules and admits that peer-review is a better guarantor of progress than automatic share-the-wealth funding and supervision by lawyers-turned-congressmen, American agricultural research policy will remain firmly in the past century. Meanwhile, the brightest young molecular biologists will continue to view agriculture as unattractive and to make their careers in bacterial and mammalian genetics.

Next year, when Congress takes up renewal of the farm bill, is an obvious moment for the would-be reformers to bring the debate on competitive-grants funding out of Jamie Whitten's back room. A fistfight on the House floor might at least make a few people sit up and notice. □

US nuclear information

Opposition to proposals for restricted access

Washington

A PROPOSED rule that would allow the US government to keep secret even unclassified information related to nuclear weapons is meeting with broad opposition from groups as diverse as librarians, trade unions and newspaper editors.

The rule, which establishes a new category of "unclassified controlled nuclear information", or UCNI, is intended to prevent the spread of nuclear weapons or the sabotage of US nuclear weapons facilities. But at hearings called last week to receive public testimony on the proposals, the Department of Energy (DoE) was accused of having gone far beyond Congress's intent by drafting "sweeping" restrictions that could deny the general public and workers access to health and safety data, place an unreasonable burden on libraries, and shield DoE's activities from legitimate scrutiny.

Last spring, when the rule was first proposed, Stanford University protested that it would also have an "unlimited potential to chill research, teaching, and the general interchange of information" (see *Nature*, 19 May, p. 189).

At last week's hearing, some of the strongest protests were heard from librarians, who could find themselves held responsible under the new rule for limiting access to UCNI to "authorized" persons. They might even have to determine which DoE documents are likely candidates for UCNI designation. Sandra Peterson, a government documents librarian at the College of William and Mary, who spoke on behalf of the American Library Association, pointed out that publications of DoE and its predecessor agencies have been in the public domain for over 20 years in some cases and that DoE technical reports on 300,000 microfiches are on file in 36 academic research libraries around the country, 35 of which are academic research libraries.

Under the proposed rule, existing materials would not be reviewed by DoE for UCNI designation until and unless a request to read them was made by a "non-authorized" person — namely anyone who is not a government employee or contractor having a "need to know", a member of Congress, or certain state and local officials. Peterson said it would be both impossible to enforce this provision, since government documents are filed on open shelves for the most part, and repugnant to the basic principle of equal access to all users.

A number of witnesses made the point that adequate measures are already

available to safeguard sensitive information. In addition to seeking national security classification, DoE can itself declare sensitive information to be "restricted data" under the Atomic Energy Act or can withhold it under exemptions provided by the Freedom of Information Act.

Rear Admiral Thomas Davies of the Nuclear Control Institute said that Congress never intended UCNI to become a "catch-all" and explicitly instructed DoE to impose the "minimum restrictions" necessary. The breadth of DoE's proposed rule, he said, creates the risk that UCNI designation will be invoked to "avoid potentially embarrassing situations"; yet public revelations of DoE's deficiencies in its safety procedures and safeguards have

prompted the agency to improve its security procedures.

Similar concerns about preserving public scrutiny were voiced by the American Society of Newspaper Editors and environmental groups. The Oil, Chemical and Atomic Workers International Union added that the rule may restrict access to health and safety records, a right guaranteed in principle by its collective bargaining agreements.

DoE's deputy assistant secretary for nuclear materials and defence programmes, Charles Gilbert, tried to allay some of these concerns. He said that only "a relatively small" amount of information would actually come under the rule. He said DoE was considering either an exemption for non-governmental libraries or that they should only have to remove documents identified by title in a written notification.

DoE can now either issue a final rule, without offering further opportunity for public comment, or can start again with a new proposed rule. **Stephen Budiansky**

European molecular biology

Enter one, exit another?

ALTHOUGH the question of Britain's continued participation in the European Molecular Biology Laboratory (EMBL) at Heidelberg in West Germany will remain in the balance at least until October, some comfort is being found in the decision of Greece to pitch in with the ten countries that already pay for the laboratory. The problem is that the Greek contribution will be only about one-tenth of what the British at present contribute and that the rot might set in if Britain did pull out.

The possibility of British withdrawal stems from a suggestion last November by the Advisory Board for the Research Councils to the Medical Research Council (MRC) to "review" its support of EMBL. Last year that amounted to £0.9 million, slightly less than 1 per cent of the total MRC budget.

The British delegation to EMBL spent three days late in July visiting EMBL and its outstation at the Institut Laue Langevin in Geneva and is now preparing its report which is likely to come before the next council meeting in late October.

The ultimate decision will have to rest on a mixture of scientific, financial and political decisions. In scientific terms EMBL has somewhat changed direction in the past year under its new director-general Dr Lennart Philipson and is now exploring areas that are more fashionable than before but, by the same token, are of the kind being undertaken in many national laboratories of the same size.

The financial position is more complex. EMBL is often accused of being well heeled compared with most national laboratories and particularly those in the United

Kingdom. There is no doubt that salaries are relatively high — good enough, says Philipson, that scientists are not forced to supplement their salary with consultancies. And one simplistic calculation is that the MRC Laboratory of Molecular Biology supports about the same number of people on not much more than half the total expenditure of EMBL. Philipson, however, points out with some justification that EMBL has to pay many benefits that the state pays in Britain and that it has well above average capital investments in the development of instrumentation, particularly for the outstations which are widely used by visitors.

While optimistic about the British decision and wishful that the British contribution would become independent of MRC, he is very concerned about the possibility of British withdrawal, particularly because it would be bound to make other countries think again. Already he has encountered some hesitancy among possible recruits to EMBL because of the cloud on the horizon.

Philipson is naturally glad that Greece will be joining EMBL from the beginning of 1984. But being a country with a low gross national product and having pleaded successfully for a cut price (one-third off) Greece will only contribute about £0.1 million per annum. Philipson hopes to persuade other countries that subscribe to the European Molecular Biology Organization but not to EMBL, particularly Finland and Norway, to follow the example of Greece. He will be hard pushed if Britain decides to drop out.

Peter Newmark

Cell culture media

Contamination at Flow Labs

BIOLOGICAL researchers throughout Europe will return from their summer holidays to find some bad news on their door-mats. Flow Laboratories Limited, of Scotland, one of the major suppliers of liquid cell culture media, have notified 179 British customers that media supplied between March and May of this year may have been contaminated with the mycoplasma bacterium *Acholeplasma laidlawii*, better known as a contaminant of tissue cultures introduced from biological sources. In mainland Europe some customers have been informed and others are in the process of being traced. Twenty-five batches of 18 culture medium types are affected, representing about 12½ per cent of the company's sales over the period.

The company first discovered the bacterium in its own tissue cultures in March, but did not suspect the liquid medium as the source of contamination. A company spokesman said it had been slow to trace the source because "at first we simply couldn't believe it". Problems with *Acholeplasma* usually arise from contaminated fetal calf serum or through the use of mouth pipettes, but in this case the water supply was responsible: the company says its water supply has deteriorated in quality during the past year.

Flow Laboratories emphasizes that the decision to notify customers was taken as soon as the test had been confirmed by an independent laboratory, even though no complaints had been received. The considerable delay between the first detection of contamination and notification was due

to the hundreds of batches which had to be tested and the need for independent verification. The company says its water was purified by deionization, but that since the contamination problem was detected a stage of reverse osmosis has been added. In addition, filter pore sizes have been reduced from 0.2 µm (considered adequate to remove most bacteria) to 0.1 µm and all batches are now being tested specifically for *Acholeplasma*. This effectively ensures that further contamination cannot occur, although the company admits it could be seen as "shutting the stable door after the horse has gone in a big way". One competing manufacturer of culture media commented that testing for mycoplasma in culture media is not standard practice, but contamination would have been impossible had Flow distilled its water.

For customers who received contaminated media, the problems are most likely to be with long-term cultures, from which experimental results will be, to say the least, questionable (or according to one scientist, "totally useless"). Most of the affected media have been used in pure research, although it seems possible that some were used for virological diagnosis. The company is offering to replace all contaminated stocks free of charge, and says many customers have responded gratefully to this offer, although the majority has not yet replied. Some individuals are considering seeking compensation as well as replacement of the affected media by Flow laboratories.

Tim Beardsley

Biotechnology

Genetic patents, the old

Washington

THE Cohen-Boyer patent, for the basic process of genetic manipulation of bacteria, was thrust back into the limelight with the filing of an unusual petition seeking to reopen to public view the Patent Office's action on the pending application. Although patent applications are by law confidential, Stanford University and the University of California originally waived their right to secrecy. Then, in the wake of unfavourable publicity over several potential flaws in the patent application last year, and the Patent Office's tentative rejection of the application, Stanford abruptly ordered that the file be closed to the public, citing "erroneous public impressions" that had resulted (see *Nature* 300, 568; 1982).

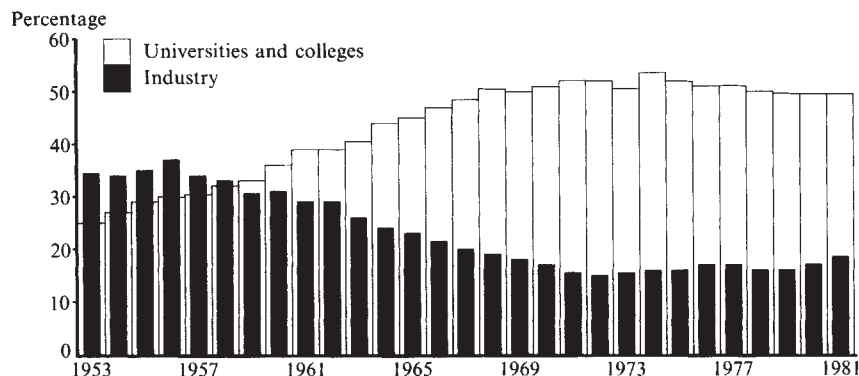
The issue has become an extremely touchy one for several reasons. Seventy-three companies have already paid Stanford a total of \$1.4 million in licence fees on the first Cohen-Boyer patent, which was granted in December 1981 and which covers the basic process for inserting foreign DNA into bacteria. The potential flaws in the second patent — the one now pending, which covers the transformed bacteria — could invalidate the first patent as well. Licensees have been chary of continuing to pay \$10,000 a year in fees to Stanford; other companies are uncertain about whether to take out licences. And although Stanford promised to keep its licensees informed of the progress of its pending application, licensees have reportedly not been permitted to keep copies of the "office actions" issued by the Patent Office. These documents frequently raise objections to the claims in a pending application and give an indication of which way the Patent Office is leaning.

The petition filed last week to reopen the process to public view came from a Washington law firm, Wegner & Bretschneider, which has represented a number of biotechnology companies in patent matters. Barry Bretschneider would not identify who he was representing in filing the petition, nor even whether his client was a licensee of the first Cohen-Boyer patent. He did note the dissatisfaction that licensees have expressed over not being kept fully informed and said that Stanford and the University of California "must only have had one motive in mind [in closing the file], namely to cover up something going on here".

Bretschneider said the only justification under the law for the secrecy of a pending application is protection of trade-secret material; by giving access to the file initially, Stanford had already exposed any such information. "You can't put Humpty-Dumpty back together again", he said.

Bert Rowland, the attorney representing

Basic research in the United States



SPENDING on basic research by industry and by academic institutes in the United States between 1953 and 1981 as a percentage of total support for basic research.

In terms of 1972 dollars, spending on basic research by industry has remained roughly constant since 1960, with a small peak of around \$215 million in 1965-67. In contrast, spending by universities and colleges has risen dramatically from less than \$200 million in 1953 to more than \$1,600 million in 1981.

In 1981, US basic research was supported by the federal government, largely through the universities and colleges (67.8 per cent), by industry (17.3 per cent), by the academic research institutes themselves (9.8 per cent) and by other non-profit making institutions (5.1 per cent). Source: *Trends to 1982 in Industrial Support of Basic Research, National Science Foundation Special Report.*

Melanie Kee

Stanford in the case, said the petition presented "a novel idea", and noted that frequently patents have been issued and simultaneously continued with applications for additional claims, and that even though the continued application contains identical "trade-secret" material, it is not subject to disclosure. And he suggested that Wegner & Bretschneider may have been motivated by "crass commercialism", since they were at one point planning to publish a book on the Cohen-Boyer patent, a plan that many have been foiled by the file having been closed.

Stephen Budiansky

... and the new

NEW YORK'S Columbia University was last week granted a patent that is widely viewed to be of sweeping importance to the future commercial production of proteins from mammalian cell cultures. The process patented was developed by Dr Richard Axel and his colleagues. It enables two new genes to be simultaneously placed and activated in mammalian cells. One acts as a selectable marker, the other produces a protein that is under study or of commercial value.

The ability to introduce selectable markers is a key to the commercial use of mammalian cells in biotechnology applications. The markers allow easy isolation of the transformed cells.

Mammalian cells will probably be used to produce proteins that are toxic to bacteria or unstable in bacterial systems; mammalian cells offer in addition the substantial advantage of being easily induced to secrete their protein products. Thus, unlike bacterial cells, mammalian cells do not need to be destroyed to harvest the product. The ability to maintain stable lines of protein-producing cells also allows for manipulation of environmental factors to optimize production.

Columbia's Office of Science and Technology is discussing licensing of the patent with several companies. Columbia, which was assigned the patent by the researchers, has said that it will grant non-exclusive licences to "facilitate the transfer" of the technology as rapidly as possible. No details on terms of the licences have been announced as yet.

The research on which the patents are based was published in three principal papers* which have been published over the past six years by Dr Axel, Dr Saul Silverstein of Columbia and Dr Michael Wigler now of Cold Spring Harbor Laboratory.

Foreign patent applications for the process are pending in Western Europe, Japan, Canada and Australia. Two additional US patents covering related work on gene promoters and amplified genes are also pending.

Stephen Budiansky

*Wigler, M. et al. *Cell* 11, 223 (1977)

Wigler, M. et al. *Cell* 16, 777 (1979)

Wigler, M. et al. *Proc. natn. Acad. Sci. U.S.A.* 77, 3657 (1980).

British birds

Peregrines prey to nest thieves

THE Royal Society for the Protection of Birds (RSPB) claims that a registration system for captive birds of prey which was introduced into Britain last year to prevent theft of eggs from the wild is acting as a "bureaucratic rubber stamp that is condoning crime". The society believes that the system is being widely abused because of ineffective enforcement, and says that the number of eggs of rare species taken illegally this year has been noticeably higher than in previous years.

The scale of the problem is illustrated by the case of the peregrine, a bird that is particularly popular with falconers. RSPB's director, Mr Ian Prestt, says that the society knows of 72 peregrine eyries that have been plundered this year, 10 to 20 per cent of the total British nests. The estimated number of eggs stolen is slightly larger than the number of eggs claimed to have been laid in captivity and so registered under the Wildlife and Countryside Act. Mr Prestt believes that many of the stolen eggs are hatched in incubators and then registered as having been laid by captive birds.



The peregrine falcon at risk

RSPB's director of conservation, Mr John Parslow, says that the Department of the Environment is unable and unwilling to take enforcement seriously. Part of the problem is that breeders of captive birds are not required to keep detailed records of eggs laid and hatched, so that registering a bird from an illegally obtained egg is quite easy, a fact that is admitted by the Department of the Environment. The society has made suggestions for improvements and offers to help policing but has received no response. But the department says it has yet to be convinced that further measures will be necessary, although it is considering the suggestions and offers of help from RSPB.

Although some species have to be ringed in the presence of an inspector from the department in order to be legally sold, some of the inspectors are unpaid volunteers who are themselves breeders of captive birds. In other cases registration rings are simply sent through the post. Mr Parslow says that unless the law can be enforced effectively there is a strong case for a total ban on the keeping of birds of prey.

A major problem facing inspectors is that it is usually impossible to invalidate claims that a particular bird was bred from captive stock, unless an egg is found that has been marked in the wild. But the society is hoping that molecular biology will come to the rescue. The Nuffield Foundation has agreed to sponsor a one-year pilot study, to be carried out by Dr David Parkin at the University of Nottingham, of a possible method of disproving fraudulent claims of parentage. A bank of molecular probes for detecting variants in mitochondrial DNA will be built up in the hope that they will enable checks to be made on maternity through the analysis of blood samples.

Tim Beardsley

Cancer chemotherapy

New recruits

Washington

THE National Cancer Institute (NCI) is spending some \$10 million this year on a new programme which, it is hoped, will increase the number of cancer patients participating in clinical trials and at the same time keep local physicians in touch with the latest developments in cancer treatment. The money will be distributed this summer to 59 community hospitals or consortia of cancer specialists in 32 states.

The Community Clinical Oncology Program will encourage qualified community physicians to participate in NCI-supported clinical trials at medical centres and in regional clinical cooperative groups that conduct large-scale treatment studies. The programme is expected to provide an additional 5,000 cancer patients a year for clinical research.

One reason for the initiative is that the United States has become the victim of its own success in training physicians to treat cancer patients. Some 80 per cent of cancer patients are believed to receive treatment in their local communities, raising acute problems for clinical researchers who need to carry out large-scale treatment studies.

Dr Robert Frelick, one of the programme directors, cited Hodgkin's disease as an example of the problem. Current chemotherapy has proved so successful that it is being administered throughout the country, leaving a shortage of patients for clinical research centres that aim to reduce the rate of mortality even further.

Each of the 59 community programmes has to undertake to provide at least 50 patients a year who are willing to participate in the research protocols developed by the NCI through affiliated centres. NCI hopes that by upgrading the skills and knowledge of local physicians even those cancer patients who are not participants in clinical trials will receive some benefit from the programme.

Peter David

Synchrotron radiation

Bright news for Daresbury

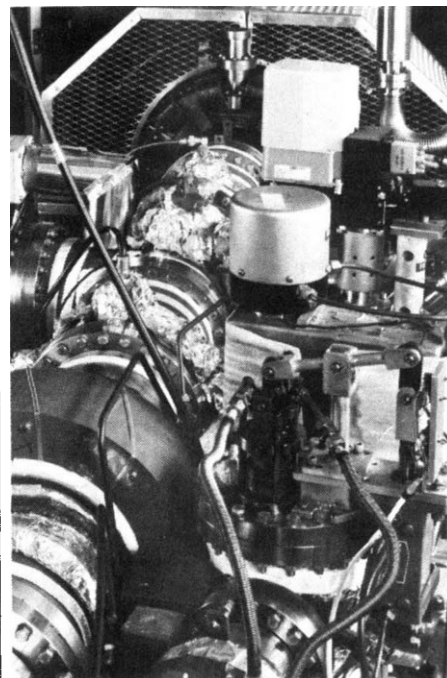
THE British Synchrotron Radiation Source (SRS) at Daresbury, Lancashire — arguably the world's best X-ray machine, used for analysing the structure of molecules and materials — is to get brighter. It will thereby remain competitive with planned American and Japanese sources. The conversion will take some five months and will cost £850,000 (to be provided by the Science and Engineering Research Council). Work is scheduled to begin in the autumn of 1985.

Meanwhile Daresbury, flushed with the success of a new wiggler beamline which has already doubled brightness, is planning to run the SRS non-stop instead of with an eight-hour break each day. In the course of the past year Daresbury has managed to double, to 18, the number of experiments which can run simultaneously on SRS; even so, user demand is still not satisfied, according to the director Dr D.J. Thompson.

Technically, the objective of the SRS improvement is to get more light out of a smaller area — to make the source look to the experimenter like a brilliant point of light. The more brilliant and point-like the source, the more information can be glean-

ed from any experiment which requires high resolution in space and time. These tend to be the experiments at the "cutting edge" of synchrotron radiation science, says Dr John McTague, the director of Daresbury's American rival, the National Light Source (NLS) at Brookhaven, Long Island: experiments such as X-ray holography and X-ray microscopy. Simple microprobe analysis and Bragg scattering are also improved by more, finely-focused light. And since the source of light in a synchrotron source is a curving beam of electrons, the experimental objective means narrowing the beam cross-section and increasing the current: a piece of accelerator technology.

Since some synchrotron radiation experiments do not need high brightness, but merely good integrated flux, there has been a degree of controversy at Daresbury over whether the five-month delay and the technical risk of brightening the source is worth it. The technology of the improvement is not simple: NLS is having troubles in commissioning its own high brightness X-ray source. (A smaller ultraviolet machine is now working well, says McTague.) The NLS X-ray ring is designed



To improve the Synchrotron Radiation Source, Daresbury engineers must find space to fit an extra quadrupole magnet in this crowded area.

Station log book

THE following entries were discovered in the log book kept at one of the experimental stations at the SRS. Dating from September 1982, they record the actual experiences of a user who prefers to remain anonymous.

Thursday, 16th

00.00 hrs. We have beam in single bunch. Spent all night aligning camera. Got tired — left at 7.10 when beam stopped.

12.00 — got beam again. Worked all afternoon. Achieved superb alignment at tea time — and started experiment but you won't believe this — THE BEAM stopped about 5.30 pm! Went for tea.

10.30 pm — Hooray. Beam again, but it's in the wrong place, waiting for bumps. Have been promised beam continuously until 10 am. Looking forward to getting results!! Got bumps at 11.00. Started experiment. 2 GeV 7.5mA.

Friday, 17th

3.00 am — everyone gone except us. Feet hurt like crazy, but ring still going, down to 4.4mA. Films look good — might actually get results this time. Ian in good spirits — amazing after only 2 hours sleep.

6.00 am — Still beam — will it never end. Despite what Ian says about sleep deprivation experiments, I don't think you can do without sleep. I feel awful.

7.00 am — 3.8mA in ring.

7.30 am — Went for breakfast, was starving. Came back 8.00 am — changed specimen but too knackered to work anymore — scared of knocking camera. Went to bed.

4.15 pm — Return, like giants refreshed. Lots of beam 2 GeV 217 mA, but it keeps going on and off.

7.00 pm — Paul Quinn drops by. Says he's given up. Whoopee, continuous beam from now on. Got great photographs. Except for the last one which was blank cause beam dumped.

Saturday, 18th

9.00 am — Back again! 2 GeV 270mA. Wow! Never get our exposures short enough. Crazy this synchrotron radiation — half the time you don't have enough beam and half the time too much.

11.07 am — Knew it was too good to last: beam gone. Gone for coffee.

12.00 — Went to control room. Found them hitting the machine and swearing. Suggested sacrificing two chickens and a goat. Gone for lunch.

1.30 pm — Back from lunch. Beam again. 2 GeV 217mA.

11.30 pm — Beam dumped. Feet now dead. Can't wait to change socks.

Sunday, 19th

Beam ran all day (more or less). 2 GeV 200mA. Took about 55 photographs. Can't keep this up much longer; thank God we've nearly run out of film.

Monday, 20th

9.00 am — Back again! Beam 2 GeV 220mA. Finished Laue experiment — ran out of film and specimens. Bliss! Might be able to get some sleep now. Feet still very bad.

Tuesday, 21st

2 GeV — 219mA. Good beam all day.

Wednesday, 22nd

2 GeV — 230mA. Good beam all day!

to have a "figure-of-merit" (involving brightness and focus) 100 times better than Daresbury's present value although it is nowhere near design value yet. Daresbury's modification should bring it within a factor of 2–5 of the NLS design.

According to McTague, increasing effort is going into commissioning the X-ray ring. Teething troubles in commissioning new accelerators — synchrotron radiation sources are essentially electron accelerators — are common in high-energy physics, but the commissioning of a synchrotron light source is even more complex, McTague says. However, he believes, "there are no unique problems" and "the notion of high brightness is OK".

McTague expects sufficient beam for the first experimenters to be able to align their experiments "in late fall", and useful currents a few months after that.

Meanwhile Daresbury is taking a close interest in the NLS commissioning, and has its principal engineer there to make sure that the Daresbury plan does not need modification in the light of NLS experience.

Meanwhile other sources in Germany and Japan are now being commissioned: in Hamburg, a modification to the DORIS electron ring (DORIS II) should give it roughly equal "figure of merit" to the SRS (unmodified), although it has not yet reached target brightness; and the Japanese "photon factory", now coming on line, is aiming for merit figures which would match the modified SRS.

Plans for a European Synchrotron Radiation Source, 1,000 times brighter even than the NLS design, are also still being pursued.

Robert Walgate

US industrial research

Incentives for innovation

Washington

WITH fear of Japanese competition assuming epidemic proportions in the United States, the Reagan Administration is mixing together a cocktail of legislative and fiscal tonics designed to persuade industry to spend more on research and development. Ingredients include a battery of tax rewards for companies increasing their research effort; major reforms of the antitrust laws; and new schemes to make it easier for corporations to raise money for high-risk research projects.

So far the United States has nothing to compare with Japan's "mighty MITI" — the Ministry of International Trade and Industry credited by American government officials with almost magical powers to target and dominate new high technology industries. But the US commerce department does have an office for Productivity, Technology and Innovation (PTI) whose head, former industrialist Bruce Merrifield, believes he can give MITI a run for its money.

One of Merrifield's main innovations is a system of Research and Development Limited Partnerships (RDLPs) through which big corporations enjoy a variety of tax advantages by raising outside venture capital to fund large-scale research projects. Under RDLPs, corporations raise money from external partners who shoulder the financial risk of the research

project in return for tax benefits, royalties and other forms of profit sharing.

The Department of Commerce says that the scheme has generated massive interest and that major RDLPs are being formed in areas such as biotechnology, semiconductors, aerospace and education technology. In a typical example last December, Genentech raised \$56.6 million from private investors for clinical testing of a new human growth hormone and



gamma-interferon. And the Semiconductor Research Corporation, the research co-operative formed last year by a dozen electronics companies, is considering using an RDLP to finance development of a new 4-megabit chip.

Merrifield's PTI also claims the credit for having prodded the Reagan Administration into crafting a package of reforms that would free companies which band together for research projects from fear of prosecution under the antitrust laws. The administration has recently been heaping praise on the Microelectronics and Computer Technology Corporation (MCC), the joint research venture set up by 12 companies to beat back the Japanese challenge in microelectronics. But MCC went ahead only because it was given a cautious green light by the antitrust division of the Justice Department.

To allay the fears of companies wishing to follow MCC's example, the administration has asked Congress to change the antitrust law so that joint research agreements will not be considered automatically unlawful in antitrust suits and will therefore not be in danger of having to pay treble damages. Furthermore, courts would in future have to weigh the erosion of domestic competition caused by such agreements against the benefit they might have for the nation's competitiveness.

PTI's initiatives are taking place against a background of lucrative tax breaks for companies expanding their stake in research. Under the Economic Recovery Tax Act companies which increase their

Biotech teams up with China

Washington

BIOTECH Research Laboratories, a Maryland-based developer and supplier of monoclonal antibodies, has signed what it believes to be the first biotechnology research agreement between a US company and the People's Republic of China.

Thomas Li, Biotech's president, told *Nature* that under a three-year contract beginning in October the company is to train members of the Shanghai Cancer Institute in the development of monoclonal antibodies at the company's Maryland Laboratories. On their return to China, the Shanghai staff would develop their own projects, with each partner permitted to use the end products. With China's new policy of encouraging foreign investment, the research collaboration could lead to a decision by Biotech to manufacture its products in China instead of importing them.

The arrangement has two obvious benefits for Biotech, a ten-year-old company that reported revenues of \$1.5 million last year. It will give the company access to clinical material from patients with diseases, such as oesophageal and nasopharyngeal cancers, which are prevalent in South-East Asia but rare in the United States. And it could give the company a foothold in a huge market for its diagnostic products.

Peter David

spending on research over a three year average qualify for a 25 per cent tax credit. PTI is arguing that the tax credit should be extended beyond its 1985 expiry date and looking at proposals to enlarge it so that the development of computer software — not currently defined as research — could qualify for tax relief. The act might also be changed to enable new companies to become eligible.

As a final inducement to companies to do more research the federal government is liberalizing its patent policies so that private firms doing research at federal laboratories can retain patent rights — which until now have belonged to the government. Companies can now also prevent research they do at federal laboratories from being published as long as they pay for the cost of the facilities they use.

It is too early to assess whether PTI's efforts to spur greater private investment in research will have a significant impact but the need for some government encouragement was underlined last month by a National Science Foundation warning that the spurt in industrial spending on basic research that started in 1975 began to slow down last year. From 1975 to 1981 firms expanded their basic research funding by 6.7 per cent a year in real terms. In 1982 the rate of growth plunged to less than 3 per cent, with most growth concentrated in the chemicals industry.

Peter David

More AIDS money

Washington

ONLY weeks after Public Health Service officials were once again insisting that adequate funds are available to deal with the acquired immune deficiency syndrome (AIDS) epidemic, Secretary of Health and Human Services Margaret Heckler announced that she would ask Congress to reallocate an additional \$22 million to AIDS research next year. Mrs Heckler made the announcement last week during a well-publicized visit to an AIDS patient in a New York City hospital.

The additional support, which would nearly double the planned spending on AIDS research for the next year, will come from money set aside for rural development loans and for Health and Human Services expenditures on construction and new furniture.

Representative Ted Weiss (Democrat, New York), whose House of Representatives subcommittee investigated the federal response to AIDS in a series of recent hearings, said that although he wished Mrs Heckler's announcement had come sooner, he was "glad that the Administration has reversed its earlier position".

Stephen Budiansky

AIDS contact

SIR — Anne McLaren (*Nature* 30 June, p.748) claims that "contact with semen may promote immune suppression" and implies that such contact may cause AIDS.

On the contrary, studies on the use of barrier contraception during heterosexual relations indicate increased breast cancer risk in women. This implies that contact with semen may actually be immunopotentiating, and help to prevent the development of breast cancer. Whatever the case, the important point is that women who contact semen during sexual relations do not become immunosuppressed and do not develop AIDS. Also, vasectomized men who form antibodies to their own sperm also do not develop AIDS.

Nevertheless, it certainly would be helpful to suggest, as Anne McLaren does, that male homosexuals use condoms to prevent transmission of possible AIDS agents. However, I don't think it scientifically objective to imply that contact with normal semen causes AIDS.

LOUIS DeTOLLA

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SIR — If, as noted recently (*Nature* 30 June, p.748 *et seq.*), contact with semen may promote immune suppression in humans and mice, why do heterosexual women and reproducing female mice not suffer from immune suppression? Or is it specific to males?

A. SIBATANI

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ANNE McLAREN REPLIES:

SIR — The immune suppression reported by Hurtenbach & Shearer (*J.exp.Med.* 155 1719; 1982) was induced by spermatozoa introduced into the blood stream. Only male mice were tested but, as Sibatani implies above, it would be interesting to know if the effect is less marked in females. Alternatively, the female reproductive tract may have evolved mechanisms to minimize the entry of spermatozoa or sperm antigens into the blood stream, to guard against induced infertility and also perhaps AIDS. A slight degree of immune suppression, at least to minor histocompatibility antigens, does appear to be associated with exposure of female mice to spermatozoa in the course of sterile matings (Lengerova & Vojtkova, *Folia Biologica* 8, 21; 1962). While we are still ignorant of what causes AIDS, any possible causative factor is surely worth investigating, and any possible form of protection is better than none.

ANNE McLAREN

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Guidelines to radiation doses

SIR — The news item "US academy sets guidelines" (*Nature* 26 May, p.275), states that the National Academy of Sciences Report "for the purposes of its own study . . . selects 10^{-4} sieverts per year as the maximum acceptable average lifetime dose rate". May I correct that misleading statement? You will find on page 216 of the academy report the following:

"The panel wishes to make clear that the individual-dose criterion of 10^{-4} Sv/yr is *not* intended as an upper limit of radiation exposure. It is simply a goal against which a particular repository system performance can be compared. The dose value of 10^{-4} Sv/yr is sufficiently low so as to provide reasonable assurance that no member of the public will be exposed to a radiation risk greater than that experienced and permitted from natural sources in day-to-day life. Other, higher limits (International Commission on Radiological Protection 1979) should be used to evaluate the upper levels of exposure estimated from the uncertainties inherent to the parameters used for calculating system performance."

Furthermore, pages 212–216 (Section 8.2) give a discussion as to how the panel arrived at a criterion of 10^{-4} sieverts per year and pages 216 and 217 (Section 8.3) discuss whether or not the value of 10^{-4} sieverts per year may be considered to be "as low as reasonably achievable".

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Pity the publisher

SIR — I quite agree with A. J. Dessler (*Nature* 14 July, p.110). The authors of journal articles do not generally want protection from copying. It is the publishers who need the protection. They are the ones who are involved in the ever-increasing cost of producing journals, and who are seeing their subscription income continually eroded by large-scale photocopying. It is therefore reasonable that the party who is most interested in copyright protection should hold the copyright.

With regard to the burdensome administration of permissions, publishing organizations worldwide are now trying to set up licensing arrangements by which copying can be done with the minimum of formality and through which reasonable royalties can be paid to the copyright holder. Unfortunately they are meeting with a lot of opposition.

J. D. ST AUBYN

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How to swat flies

SIR — The potential for house flies to spread disease has, of course, long been recognized. A fly, having crawled over human or animal faeces, may enter the house eventually to alight on exposed food. Usually the fly, attracted by light, whizzes up and down the window. Attempts to swat it dead are usually thwarted since the fly has a high-speed (millisecond) reflex system in its visual-brain-motor system so that it responds by taking off at an avoiding angle in response to a moving approaching swat entering its visual field.

In the interests of hygiene I have experimented on the most effective way of swatting. A piece of tissue paper is taken in each hand and the fly approached from the left and right, keeping the hands equidistant from the fly and moving to and fro slightly, then both hands simultaneously pounce. The fly cannot cope with this situation since its central nervous system circuitry is geared to avoid approaching movement in only one part of its visual field at a time. Two simultaneously approaching swats render the fly immobile, for its central nervous system now cannot compute at which angle to take off.

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In vitro mussel culture

SIR — We believe Young and Williams' review¹ of Roberts' article² involving our research³ missed the real point of the review. Unlike the British species *Margaritifera margaritifera* (L.) for which the fish host is evidently known, host(s) of most species are unknown. In the United States there are numerous endemic species which are extremely rare and for which there are no known host(s). Therefore, an *in vitro* culture method such as ours is the only hope for sustaining these species until a natural fish host or other host is found.

We have, and are now growing, juvenile mussels resulting from *in vitro* culture in the laboratory and in a field laboratory setting. It appears entirely feasible to apply our culture system to assure conservation of a species like *M. margaritifera*, although we would fully agree that natural expansion of populations would be desirable, if possible.

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1. Young, M.R. & Williams, J.C. *Nature* 302, 372 (1983).

2. Roberts, R.J. *Nature* 302, 13 (1983).

3. Isom, B.G. & Hudson, R.G. *Nautilus* 96, 147 (1983).

Anatomy of a price war

R.P. Haining*

The development of a petrol price war owes much to the structure of petrol retailing and in particular to the spatial pattern of retail outlets. Local neighbourhood, retailer competition, stimulated by consumer price sensitivity with respect to clusters of service stations, influences not only the spatial pattern of prices but may also influence the range in prices during a price war, at least at the scale of an individual city.

FROM the end of 1981, throughout the early months of 1982 a persistent petrol price war was widely reported in the media. In mid-January 1982 newspaper reports were documenting the price slide and the reasons for it, noting reasons for the overall national decline in petrol prices and also observing broad regional variations in average prices¹. Such average price falls conceal considerable variation and this is certainly evident at the scale of individual urban areas — the scale at which most purchasers hunt for their petrol. This note outlines some findings from a study of petrol price variation within a single urban area (Sheffield). The purpose was to identify reasons for intra-urban price differences, explore the importance of neighbourhood competitiveness on price levels and in addition identify changes in the anatomy of the price surface as the war intensified in the first three months of 1982. The results confirm the importance of neighbourhood competitiveness and identify a tendency for price variation to decline, with competitiveness being concentrated at the low end of the price scale as the war intensified.

The most frequently cited explanations of spatial price variation emphasize the existence of discrete market regions each with their own local supply and demand functions. Price differences set up flows of exports and imports between regions which result in a spatially competitive equilibrium price level which is dependent on transport costs between the markets².

But price variation exists at many scales that almost certainly do not reflect either transport costs or the existence of spatially discrete market areas. In the case of petrol sales, market interdependence exists not only by virtue of the mobility of the consumers but also the spatial price awareness of sellers *vis-à-vis* their competitors be, these company retail outlets or independent traders. A simple model of intra-urban petrol pricing would recognize such inter-market dependence in terms of the demand function at each outlet. That consumers display such price sensitivity even over quite small price differences has been confirmed by independent research³.

Let D_t denote the demand vector at time t . The vector is of length n , each entry referring to an individual retail outlet. Then let S_t denote the n -dimensional supply vector

at time t . Then assume

$$D_t = Ap_t + c \quad (1)$$

$$S_t = Bp_{t-1} + e$$

where p_t and p_{t-1} are the n -dimensional price vectors at times t and $t-1$ respectively. The vectors c and e represent vectors of constants. It is further assumed that the matrix B is diagonal, that is, the supply of petrol is scheduled and it is for the local agent to ensure that his 'market' is cleared. The diagonal elements of B are positive. A , however, is a non-diagonal matrix, the individual elements representing the degree of inter-market dependence. The diagonal elements of A are assumed negative and the

off diagonal elements positive.

Finally we assume that clearance must take place in each market independently so that

$$D_t - S_t = 0 \quad (2)$$

From (1) and (2) it follows that

$$p_t = A^{-1}Bp_{t-1} + A^{-1}(e-c)$$

and that the equilibrium price vector (p_e) is

$$p_e = (A-B)^{-1}(e-c) \quad (3)$$

an equilibrium that is stable providing the eigenvalues of $A^{-1}B$ are less than 1 in absolute value.

Rearranging terms in (3) we obtain

$$p_e = (B^{-1}A)p_e + B^{-1}(e-c) \quad (4)$$

Splitting the $(B^{-1}A)$ matrix into diagonal and off-diagonal elements we obtain from (4) the equilibrium price for the i th outlet:

$$p_{i,c} = \sum_{j=1}^n \frac{a_{ij}}{b_{ii}-a_{ij}} p_{j,c} + \frac{c_i-e_i}{b_{ii}-a_{ii}} \quad (5)$$

where the (a_{ij}) and (b_{ij}) reference elements in the A and B matrices respectively. We note from (5) that $a_{ij}/(b_{ii}-a_{ii})$ is always positive.

The model (5) suggests the presence of mean variation in retail petrol prices (the second term in (5)) and spatial contiguity — that is the tendency for neighbouring retail outlets to charge similar prices (the coeffi-

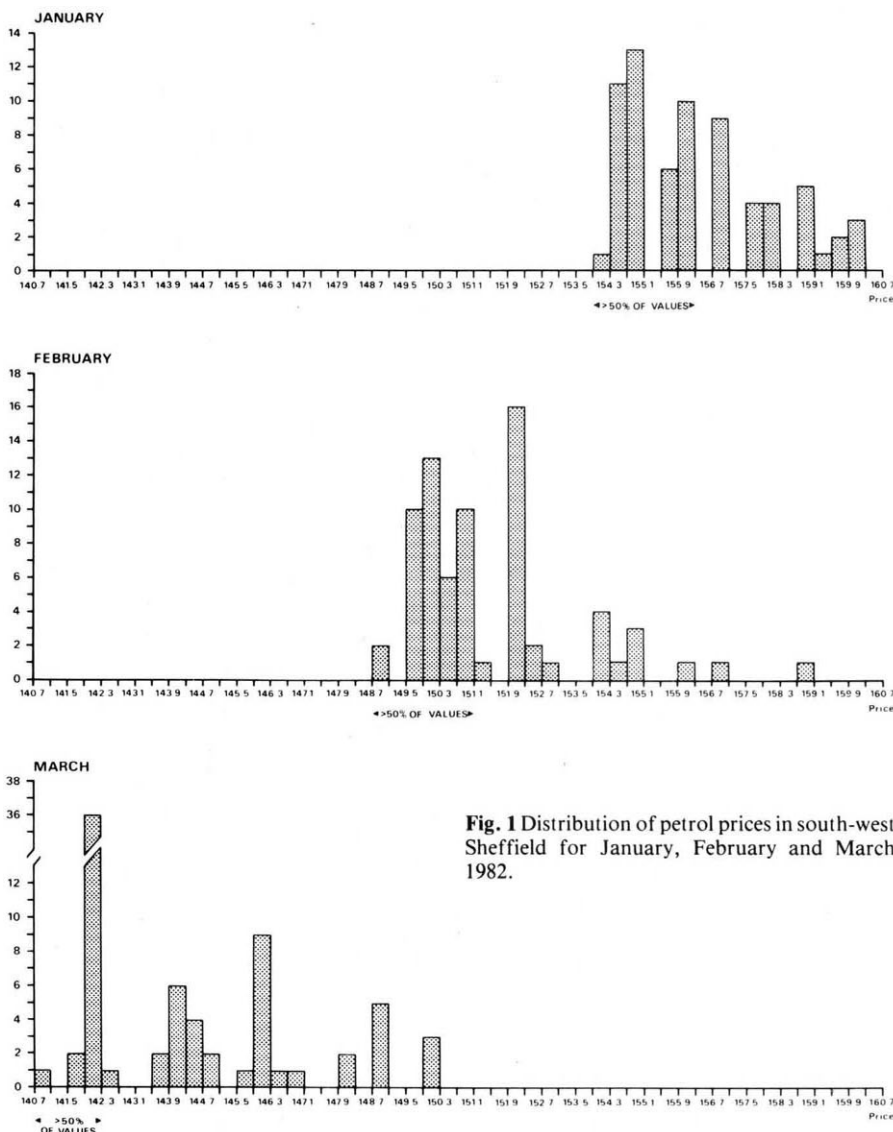


Fig. 1 Distribution of petrol prices in south-west Sheffield for January, February and March 1982.

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Table 1 Competition parameters for various petrol price ranges

	Price range		Competition parameter	log likelihood difference
January	153.9	156.0	0.80	4.06
	155.0	156.0	0.47	1.19
	156.9	157.0	-0.35	0.47
	157.8	158.0	0.97	1.11
	158.7	160.0	1.52	4.90
February	148.7	151.0	0.72	3.24
	148.7	152.0	0.97	5.31
	150.5	151.0	0.49	2.92
March	140.9	142.1	1.10	10.00
	142.0	142.0	1.14	2.90
	145.9	146.0	0.11	0.01

cient in the first term in (5)).

One other implication may be noted. Suppose in (1) it is assumed that

$$D_i = Ap_i + c + u$$

where u is an independent normal random variable so that the demand function is stochastic — a not unreasonable assumption given the high degree of mobility and the variable timing of individual consumer demand. If the slope of the demand function steepens (as it might be expected to during periods of stiff price competition when consumers become more price sensitive) then it follows that the variation in equilibrium prices (derived from the probability distribution of cutting points of the supply and demand functions) will decrease.

Data for 85 petrol stations in south-west Sheffield were collected on single days in January, February and March 1982. Advertised prices were recorded for four-star petrol and in addition the brand name was recorded together with whether the outlet also did repair work, sold cars and whether it was located on one of the principal routeways of the city of Sheffield.

A multiple regression model was run, regressing observed prices on three dummy variables (location, repair work and car

sales) and two variables that identified the (x, y) coordinates of the station. The latter two variables (obtained by digitizing the map of retail outlets) were designed to pick up trend effects. In all three months the location variable (whether the petrol station was on a principal routeway or not) was significant. The partial regression coefficient suggested an average price mark up for those stations off the principal routeway of between 3 and 4 pence per gallon. In January the repairs dummy variable was also significant giving an additional 2 pence per gallon mark up. No other variables were significant. In all cases, however, the level of explanation attained was under 10%.

Figure 1 shows a frequency plot of the prices for each of the three months. During this period prices were falling — the minimum in January was 153.9 pence, by March it was 141.0 pence. During this time not only was the distribution of prices bunching increasingly around the low competitive price level but, perhaps because of the need to preserve market share, the minimum price level charged by each brand was also converging. In all three months, all companies were represented in the spread of prices but whereas in January

there were considerable price differences in company minima these had more or less disappeared by March.

Price competitiveness was studied at a variety of levels and not only at the low end of the price range. The intention was to see to what extent spatial price competition was maintained at all levels of pricing as the price war intensified.

Interaction models were used to test for competitiveness⁴⁻⁷. Early applications of these models was to crop yields and the study of inter-plant competition^{4,8}. Here petrol stations in the price range to be investigated were coded one and the rest coded zero. The auto-logistic model was then fit using a pseudo-likelihood estimation procedure for the two parameters α and β ^{6,9}.

Estimation for each price range (t_1, t_2) proceeds by maximizing (numerically) with respect to α and β

$$\prod \frac{\exp[(\alpha + \beta y_i)x_i]}{1 + \exp[\alpha + \beta y_i]}$$

The product is over all sites that have links and

$$x_i = \begin{cases} 1 & \text{if the } i \text{th station charged in the price interval } (t_1, t_2) \\ 0 & \text{otherwise} \end{cases}$$

Then

$$y_i = \sum_{\substack{j=1 \\ j \neq i}}^n w_{ij} x_j$$

where w_{ij} is 1 if site j is a neighbour (linked) site of site i and is zero otherwise. (Unlinked sites are excluded from the analysis.)

The second parameter (β) is a measure of map structure, a positive value implying contiguity in the distribution of values. An approximate test of significance using the $\frac{1}{2}\chi^2$ distribution was used taking the difference in the log likelihoods for the case $\beta = 0$ and the case $\beta \neq 0$ (ref. 9).

A variety of different price ranges were investigated and some of the results are recorded in Table 1. The approximate critical value for the test is 1.92 in each case. The linkage or edge system imposed on the sites reflected first the orientation of the principal Sheffield routeways, and second, proximity, where groups of stations were close together but not actually on the same routeway. Thus the measures of competitiveness are principally with respect to proximity on or close to the main urban routeways (Fig. 2).

The evidence suggests that competitiveness increased at the low end of the price range as the price war intensified. Equally, however, perhaps because of the increasing price sensitivity of consumers, competitive interaction at prices well above the area minimum declined. □

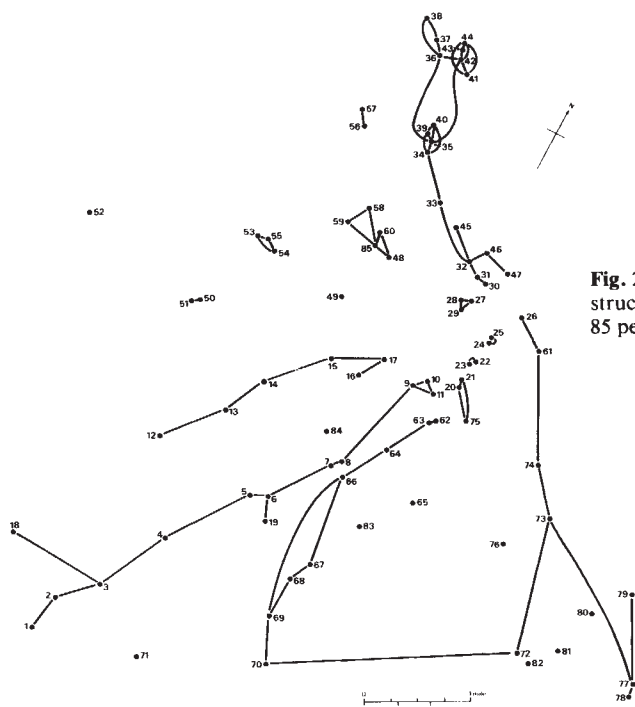


Fig. 2 Imposed edge or linkage structure for the set of 85 petrol stations.

1. *Sunday Times Business Supplement*, 24 January 1982.
2. Samuelson, P.A. *Am. Econ. Rev.* **42**, 283-303 (1952).
3. *Sunday Times* 20 June 1982.
4. Whittle, P. *Biometrika* **41**, 434-49 (1954).
5. Preston, C.J. *Gibbs States on Countable Sets* (Cambridge University Press, 1974).
6. Besag, J. *Jo. R. Stat. Soc. B36*, 192-236 (1974).
7. Haining, R.P. *Geogr. Anal.* **14**, 95-108 (1982).
8. Mead, R. *Biometrics* **23**, 189-205 (1967).
9. Besag, J. *Statistician* **24**, 179-195 (1975).

Emerging solar systems in view

The Infra-Red Astronomy Satellite has produced evidence of the early stages of planet formation around a star. It is not the only evidence of solar systems other than our own.

WITH a discovery as provoking to the imagination as a possible new solar system (will it be like ours? will it have life?) it is almost inevitable that a crop of excited and over-simplified stories will appear in the media ("Hullo out there" began one editorial in a Canadian newspaper). Just as inevitably, there will be some who will ask: why should we be excited, surely it is no surprise? So how should one react to observations from Hawaii that indicate, some say, a giant planet orbiting T-Tauri; to recent measurements with the new Japanese millimetre-wave radiotelescope at Nobeyama, near Tokyo, which seem to show swirling gas clouds around a star in Orion; and to this month's results from the Infra-Red Astronomy Satellite, IRAS, suggesting something similar (but much smaller) around the star Vega?

Certainly it is not surprising that stars probably form from collapsing gas clouds, and that in these clouds subsidiary vortices might form planets. But the theory of the process is uncertain, and it is not known how frequently planets form. Observations are needed but have been difficult for various reasons — paramount among them being that planets are small and dark; that planetary orbits are expected to be too small to resolve readily from Earth even if the planet is bright; and that a forming system is inevitably shrouded in dust. Hence there would be great delight if more planets, or solar systems in the process of formation, could be found and studied. (And such observations — if there were enough of them — would also eventually bear on the vexed calculations of the probability of life in the Universe, by increasing the accuracy with which the formation of an Earth-like planet can be predicted.)

Therefore, there is great interest in discovering solar systems at any stage of their development. In practice this probably means the formative stage, when the cloud and protoplanets should be hot and radiating. Such conditions are the basis of the recent measurements, although one should not forget the older technique, independent of temperatures, based on observing a wobble in the orbit of a star due to its planets.

Unfortunately that technique is only applicable in favourable circumstances with close stars. Such was the case with Barnard's star, the closest in the northern sky, whose tiny wobble of a few hundredths of an arc second has long been thought to indicate the presence of (probably two)

planets.

The latest measurements, however, are thought to represent direct observations of clouds, condensing or not. The most recent is the IRAS observation of Vega, which came about by chance. While Vega was being used for calibration it was observed to be 10–20 times brighter than it should be at wavelengths of 60–1,000 μm , assuming the star radiates like a black body at its optically-defined temperature of 9,700 K. The star is also extended at these long wavelengths, to about 20 arc seconds — or around 80 astronomical units (1 AU is the radius of the Earth's orbit about the Sun) at the 26-light-year distance of Vega. The IRAS team's explanation of these observations invokes a ring of dust with grains probably greater than 1 mm diameter (compared with interstellar grains of 0.1 μm) orbiting Vega. The dust would be heated to 90 K by Vega's radiation and would re-radiate predominantly at the wavelengths detected by IRAS.

Vega is a hot bright star which has a predicted lifetime of around 300 million years (compared with the Sun's 10,000 million), so the cloud can be interpreted as the "early stages" of a solar system — although Vega might be dead before the system can be completed.

The possibility of a "planet" near the star T-Tauri, one of the best examples of a very young star, or protostar, was raised shortly before the IRAS observations by R.B. Hanson, B.F. Jones and D.N.C. Lin (*Astrophys. J. Lett.* **270**, L27; 1983). They base their suggestion on observations by H.M. Dyck, T. Simon and B. Zuckerman (*Astrophys. J. Lett.* **255**, L103; 1982) of a low-luminosity infrared source near T-Tauri. When measured by Dyck *et al.* with a University of Hawaii telescope, using speckle interferometry to separate close objects, the infrared luminosity was lower by a factor of ten than the lowest luminosity infrared point source previously recorded.

Hanson *et al.* suggest that the infrared luminosity is the heat from a very large condensing protoplanet. But Zuckerman professes himself unconvinced. The controversy will probably be unresolved until there is better theoretical understanding of how much and in what wavelengths a protoplanet should radiate.

Between the Hawaiian and IRAS observations come the Japanese millimetre wave observations (still unpublished) that were interpreted as evidence of protoplanetary

disks — the condensing gas clouds in their final stages. Using fine molecular lines emitted by carbon monosulphide, a group headed by Dr Norio Kaifu claimed observations of Doppler shifts caused by the rotation of a disk around a star in Orion. But the resolution of the telescope (about 20 arc seconds) is at the moment poor by optical standards (it should improve 20-fold when an interferometer system is complete at the end of next year). So the discovered Orion disk is large (40,000 AU: the radius of our Solar System is about 20 AU) and the central star very bright and large (about 300 solar masses). Therefore the chances are this cloud will be blown away before planets can form.

The group also found other candidate disks: one around the star L1551 in Taurus was only 15,000 AU across, Kaifu says. In both cases, measurements with other techniques have shown matter streaming away from the central stars along a line perpendicular to the disk plane: this agrees with predictions that the bright protostar will easily be able to "blow away" matter perpendicular to the disk but not in its plane, where the matter is too dense.

Thus the Japanese may indeed have found the first evidence for large planetary disks, and it would be interesting if IRAS could turn to check the measurements. Unfortunately the orbital observatory does not have the spectral resolution necessary to detect disk rotation (by Doppler shift). With its high angular resolution, however, IRAS may be able to add structural and temperature measurements of the putative disks — and, conceivably, detect hotspots that might indicate the early formation of planets.

At the same time IRAS can safely be predicted to discover more candidate solar systems during its general sky survey — to be repeated three times before the observatory runs out of cooling helium at the end of the year. And the Japanese are planning to add an interferometer to their telescope to improve its resolution 20-fold.

Not to be outdone, the angular resolution of the speckle interferometer on the University of Hawaii telescope is to be sharpened. Further down the line are proposed ground-based telescopes with resolutions close to one hundredth of an arc second, offering hope of seeing planets or protoplanetary disks.

There should be much more evidence of solar systems to come in the next months and years.

Robert Walgate

Archaeology

New finds at Pincevent

from Paul G. Bahn

THE 1983 excavation season at the French site of Pincevent began recently and promises to be of extraordinary interest. In 1981 it seemed that work at the site was nearing its end, but last year's excavations produced some remarkable finds which have given fresh life to an already legendary site.

Pincevent, an open-air magdalenian encampment on the left bank of the Seine, in north France, was discovered in 1964 and, through the pioneering work of André Leroi-Gourhan and his team, became one of the most important archaeological sites in the world, not only because it served as an experimenting ground for new techniques but also because of its role as a training-school for a whole generation of archaeologists, some of whom are now excavating similar sites in northern France.

Horizontal excavations of open-air palaeolithic habitations had been carried out previously, most notably in Russia, but it was Pincevent which really opened the eyes of western archaeologists to the potential of the technique, and indeed extended that potential. Over promising surfaces, hangars were constructed, allowing protected and unhurried investigation, which often lasted for several years. Multiple recording techniques were used, including not only plans and photographs, but also latex moulds of large areas and of stratigraphies. In this way, synthetic materials preserve the real images of structures and living-areas now destroyed. From these detailed records, studies have been made of the distribution and spatial interrelationships of different types of object; of the systematic fragmentation of bone; of the dumping of refuse in particular spots; of the relationship of hearths, areas for activity and probable sleeping-areas; and, by re-assembling flint nodules, of the knapping done on the site.

A series of magdalenian occupations oc-

curred here around 9,000 BC on the sands and gravels of the Seine; they are interspersed with flood loams which preserved them after abandonment. The stratigraphy shows that people settled there at least 10 times, although the frequency and duration of the visits is unknown. Nevertheless, the homogeneity of the finds indicates that the site was used for no more than a few centuries in the late Magdalenian.

Pincevent lies near a ford over the Seine which may have existed then, and would presumably have been used by reindeer herds. Certainly the inhabitants exploited this resource, as reindeer constitute almost 100 per cent of the fauna, which is very well preserved. This has permitted some interesting insights: for example, repeated finds of hyoid-bones and upper canines at hearth-edges suggest that reindeer heads were cooked and eaten in the tents.

One intriguing discovery is that of an isolated horse tooth near the hearth inside several 'tents' and a fragment of horse bone just outside other tents. In view of the excellent preservation at the site, it is clear that this distribution is deliberate, and can be compared with the isolated horse teeth or jaws found carefully placed inside magdalenian hearths in some deep Pyrenean caves¹.

Despite the immediate proximity of the Seine, neither fish remains nor bird bones have been found.

The dentition and antlers of the reindeer point to an arrival of the Magdalenians between May and July, but their date of departure — possibly autumn or December — is uncertain. The site is rich, but does not display evidence of sufficient activity to fill a long stay (for example, art objects are extremely scarce). Moreover, rain affects the terrain badly, and the fine condition of the abandoned camps suggests short stays in the dry season. Estimates of length of stay are further complicated by the fact that

hearth-stones seem to have been re-used and moved around.

Since 1964, 20 large hearths and around 100 habitation structures have been explored on the preserved part of the site (~3,500 m², perhaps no more than one-quarter of the original). The first structure² studied is still enigmatic, and has been interpreted as a long tent of ~30 m², containing three living units, each with a hearth. These hearths, which are covered and filled with stones, differ from almost all the others in the site, which comprise a 'corona' of stones, bare in the centre. Being lower in the stratigraphy, the three filled hearths may represent a different tradition.

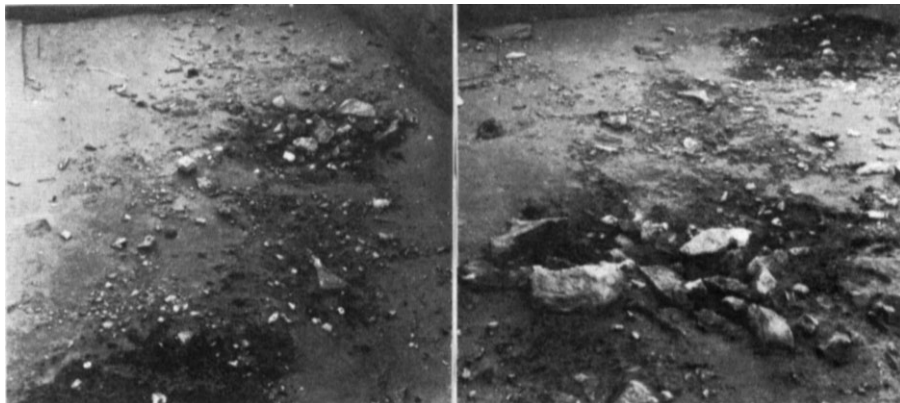
Nothing is known of the superstructure of the tents which are supposed to have enclosed the hearths and activity/sleeping areas. Reconstructions using poles and skins show that each tent probably required about 35 reindeer hides. Extensive skin-working may explain the abundance of ochre in the site: small heaps of ochre and frequent 'aureolas' of ochre round the hearths, inside the tents, have been found. Other possible explanations such as ritual floor-colouring and artistic activity seem less likely.

Such is the regularity of the structures (albeit with differences in density and richness of finds) that their layout can more or less be predicted during excavation. The tents had a circular or oval outline, ~3 m in diameter, cut by a circular hearth, ~50 cm across, in front of the entry. Thus the total surface area is about 7 m². Beyond the hearth, in the tent, was a red area containing numerous tools and at the back was an empty space interpreted as the sleeping-area. Outside the hearth, stones and bone detritus, charcoal and burnt stones were scattered up to 7 m from the tent. Clearly, flint was worked in the tents and the debris thrown outside. It is notable that burins are concentrated near the hearths, while scrapers are more widely distributed. Recent analysis of some flint tools suggests that bone-working had an important role at the site.

The description of three habitations of 'Section 36' has become a classic or archaeological publication and ethnographic analysis³; and eight other habitations should soon be published.

Last year, a new series of structures was uncovered. In 1974, a *sondage* in section 27 revealed two living-floors in level IV-4, 1.2 m below the 'classic' level of IV-2, and several other living-floors have since been discovered between the two levels. Most work has concentrated on the upper layers (IV-2), where it is now known that there are two levels of occupation, separated by only a few centimetres of sediment. Eight habitation-structures, thought to be contemporary, are known in the upper level and three in the lower.

In 1982, the removal of a hearth in layer IV-2, section 27, led to a remarkable discovery⁴: about 15 cm east of the hearth,



Pincevent: left, general view of the newly discovered stone-filled hearth and habitation in layer IV-40. View from the south. Right, the same, seen from the north. (Photographs by A. Leroi-Gourhan.)

a *baguette* of reindeer antler, 40 mm long, was stuck vertically into the sediment. Two flint bladelets, 14 and 22 mm long, were still attached, one at each side of the shaft, though the fixing-agent is now replaced by sediment. The object seems to have been placed there purposefully and to represent a weapon rather than an knife.

Moreover, a living-floor was uncovered in level IV-3 which contained large flint blades and nuclei, bigger than those of later occupations. Below, about 20 m² of a IV-4 floor were uncovered, featuring a superb habitation (see the figures) with a stone-filled hearth, 80 cm in diameter. Numerous flints (especially burins) lie on heavily ochred soil to the north and west of the hearth; to the south, ash and lithic/bone material stretch for several

square metres. The fauna consists principally of reindeer, but also includes horse, hare and even mammoth.

It looks as though Pincevent has not ceased to surprise us, and will continue to be one of the most fascinating and instructive archaeological sites in Europe for many years. □

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1. Bahn, P.G. *Les Pays de l'Audour, Royaume du Cheval*, 21 (Musée Pyrénéen, Lourdes, 1982).
2. Leroi-Gourhan, A. & Brézillon, M. *Gallia Préhist.* 11, 263 (1966).
3. Leroi-Gourhan, A. & Brézillon, M. *Fouilles de Pincevent. Essai d'Analyse Ethnographique d'un Habitat Magdalénien 7th Suppl.*, Gallia Préhist., 2 vols (CNRS, Paris, 1972).
4. Leroi-Gourhan, A. *Bull. Soc. préhist. Fr.* 80, 154 (1983).

Cell motility

Particle transport

from Dennis Bray

ONE of the most astonishing facts about the intracellular movement of membranous organelles is that we still do not know what drives them. We cannot say, at present, whether the particle we see in the light microscope, moving steadily from one side of a cell to the other, is being driven by a microtubule-based mechanism, as in ciliary beating; by an actin-based mechanism, as in muscle contraction; or by an as-yet-unprecedented mechanism based on intermediate filaments. The reason for our ignorance is quite simple: it has so far not been possible to separate the motile machinery in a functional form from the rest of the cell. No one yet knows how to break open a cell and pull out a functional transport system, free of extraneous material, that continues to carry mitochondria and vesicles from place to place. But a start has been made, and four recent papers illustrate the progress and limits of contemporary efforts. A fifth paper raises the novel prospect of starting from the other direction: of building up the transport from scratch with purified molecules.

Organelle movements in the cytoplasm do not depend on ion flux across the plasma membrane, which is important only in that it maintains the internal *milieu* of ions and small molecules. Thus, it is possible to remove the cell membrane without interrupting organelle movements provided that a suitable buffer is supplied. A recent study of rapid axonal transport — one of the most accessible and extreme forms of organelle movement — illustrates the approach¹. Single large axons were dissected from the walking legs of the lobster (with edible parts suitably discarded) and mounted for observation in a buffer containing 0.02 per cent saponin, a soapy plant glycoside. If the buffer lacked ATP then all movement rapidly ceased and the organelles became totally immobilized, not

even showing brownian movement. Addition of ATP — but not a nonhydrolysable analogue of ATP — then restored movements which continued in an apparently normal fashion for up to an hour. From this we may conclude, first, that the normal permeability barrier of the axon had been destroyed; and second, that rapid organelle movements require ATP hydrolysis. In a separate study in which the plasma membrane of crab axons was disrupted by electrical discharge rather than with detergents², movements were similarly found to be dependent on ATP hydrolysis. Interestingly, they also seemed to be independent of Ca²⁺ concentration over a wide range, implying that organelles move through the cytoplasm 'flat out', or in an unregulated manner.

A more cathartic approach is to remove the cytoplasm entirely from the cell. Years ago, Baker, Hodgkin and Shaw³ found that axoplasm could be squeezed from a giant squid axon like toothpaste from a tube. Their interest was in the empty tube, which could be perfused with buffers and cannulated with electrodes and which eventually became the doyen of electrophysiological preparations. But the blob of expelled cytoplasmic jelly appeared inert under the microscope and was therefore ignored.

Then, a year ago at the Woods Hole Marine Biology Laboratory, a group of biologists looked again at extruded squid axoplasm using a newly developed optical system. This has the endearing acronym of AVEC-DIC (Allen video-enhanced contrast differential-interference contrast) and uses the background-subtract facility of some commercially available video cameras, usually in concert with a computer image-analysis system. Astounding results have been obtained with AVEC-DIC, which allows detection of structures

far smaller than the 0.2- μ m resolving power of light microscopes. In flattened keratinocytes, for example, organelles and vesicles have been observed moving closely along linear elements subsequently identified as microtubules⁴, and described in a paper that comes with its own video disc. Moreover the extruded axoplasm, thought hitherto to be so barren of activity, is with this technique revealed to be teeming with rapidly moving small particles⁵. These are submicroscopic vesicles 30–50 nm in diameter, which travel at steady speeds of up to 10 μ m s⁻¹. Movement continues unabated for several hours, in the absence, it should be remembered, of a plasma membrane or any other permeability barrier, thereby presenting unparalleled opportunities for future experimental intervention.

Exciting though these preparations are, however, it may not be possible to continue the dissection physically or pharmacologically down to the level of the elemental motile machine. In which case, some radically different approach is required. Considerable progress has been made in recent years in the construction of motile 'models', in which large-scale movements can be produced using actin and myosin, the two proteins that generate muscle contraction. Now a model very close to organelle movement has been described in which myosin-coated beads are made to crawl along aligned bundles of actin filaments⁶. The plastic beads, 0.7 μ m in diameter, were coated with heavy mero-myosin (HMM) — the active head region of the myosin molecule — while the actin filaments were those naturally present on the inner surface of the giant algal cell of *Nitella*. Beads applied to the exposed inner surface of the giant cell attached and moved at steady rates of up to 10 μ m s⁻¹, persistently and in one direction. Movement depended on ATP and on a functional myosin ATPase activity but was insensitive to variations in Ca²⁺ concentration. Calculations showed that a single myosin molecule could provide enough force to move a bead at a rate of 5 μ m s⁻¹, and that 25 or more functional myosin heads on the surface of a bead would be sufficient to give uninterrupted movement at this rate.

Clearly an actin-myosin interaction is one way to produce organelle movement. Could this be how the cell does it, with the myosin bound to the organelle and the actin filaments closely associated with microtubules? It looks as though we may soon know. □

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1. Forman, D.S., Brown, K.J. & Livengood, D.R. *J. Neurosci.* 3, 1279 (1983).
2. Adams, R.J. *Nature* 297, 327 (1982).
3. Baker, P.F., Hodgkin, A.L. & Shaw, T.I. *J. Physiol. Lond.* 164, 355 (1962).
4. Hayden, J.H., Allen, R.D. & Goldman, R.D. *Cell Motil.* 3, 1 (1983).
5. Brady, S.T., Lasek, R.J. & Allen, R.D. *Science* 218, 1129 (1982).
6. Sheetz, M.P. & Spudis, J.A. *Nature* 303, 31 (1983).

Cell biology

The importance of the endosome in intracellular traffic

from Colin R. Hopkins

IN recent years it has become clear that all animal cells have a common mechanism for internalizing different ligands bound to the specialized receptors on their surfaces. The fate of both receptor and ligand once they have entered the cell can, however, vary between one ligand-receptor complex and another. It now seems that an important determinant of that fate is the endosome — a recently discovered organelle that occupies a central position in the intracellular traffic.

Most of what is known about the pathways involved in the receptor-mediated uptake of endogenous ligands derives from studies on low-density lipoprotein (LDL) uptake in fibroblasts¹ and asialoglycoprotein (ASGP) uptake in hepatocytes². These pathways begin at a specialized region of the cell membrane known as a coated pit, whose principal distinguishing feature is the coating of clathrin molecules on its intracellular surface. Once inside the cell, the complexes — in the case of LDL and ASGP — are separated again: the ligand is transferred to a lysosome and the receptor is returned to the cell surface for re-use.

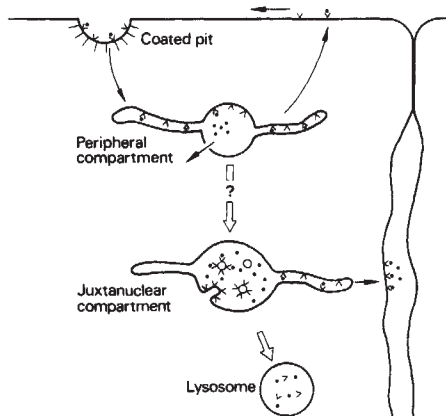
The LDL and ASGP systems are primarily concerned with delivering a continuous supply of ligand to lysosomes and essentially similar routes are known to exist for a variety of other tissue-fluid end products³. However, for ligand-receptor complexes in which the ligand, as well as the receptor, needs to be conserved there are alternative patterns of processing.

One such pathway, through which the iron transporter protein, transferrin, is shuttled back and forth across the plasma membrane, avoids the lysosome altogether. This pathway, which probably exists in all eukaryotic cells, begins with the internalization of iron-loaded transferrin by receptor-mediated endocytosis via coated pits⁴. But the transferrin remains bound to its receptor inside the cell and, having released its iron, returns, still bound to the receptor, to the cell surface where it is itself released^{5,6}. Receptor-mediated uptake with subsequent reappearance and release of ligand at the cell surface is of course the usual pattern of events in the transcellular transport of immunoglobulins and other proteins across lining epithelia and hepatocytes^{7,8}.

At the other end of the spectrum of alternative patterns of processing are systems in which both receptor and ligand are delivered to the lysosome. This results in the rapid depletion of the surface receptor population and is often called down-regulation. The best documented example

is that of epidermal growth factor⁹, but many other instances have also been described. The route from the plasma membrane to the lysosome is clearly a well beaten path; now, as different classes of receptor are being studied in the same cell, the full extent of the common pathway is beginning to emerge.

In the great majority of studies so far, coated pits have been shown to define the cell-surface site at which uptake occurs³. Much remains to be learned about the role of the pits in uptake, but from the available



In the peripheral compartment, low pH allows uncoupling of some ligands, for example asialoglycoproteins, and escape of some viruses and toxins. Some receptors, for example those for transferrin, return to the cell surface. In the juxtanuclear compartment, some receptors, such as those for immunoglobulin, are delivered to the basolateral border, while others are delivered to the lysosome and degraded.

evidence it seems that while they may act as effective filters of integral membrane proteins moving into the cell^{10,11}, their range of discrimination is low. Thus, although coated pits may be able to distinguish between membrane proteins that can enter the cell and those that are confined to the cell surface, there is so far no evidence that coated pits can select between different 'species' of migrant receptors. Indeed, several recent studies have shown different kinds of receptors within the same coated pit, and in normal human fibroblasts receptors as different as those for LDL and EGF have been found together in 75 per cent of the total coated-pit population¹².

The realization that coated pits provide a common port of entry for a wide variety of endocytosed ligand-receptor complexes suggests that the selection of which kind of processing is appropriate probably occurs intracellularly. It also greatly strengthens the probability that most, if not all, of the endocytic pathways thus far identified are

part of the same intracellular processing system.

The idea that a distinctive cytoplasmic compartment exists for the processing of internalized ligand-receptor complexes is, of course, not new (see, for example, refs 13 and 14), but until very recently it was based largely on the identification of endocytosed ligands by electron microscopy. However, in recent cell-fractionation studies on a variety of cell types, a cytoplasmic compartment with a characteristic buoyant density and highly enriched in endocytosed receptors has been isolated¹⁵⁻¹⁹. Components within the receptor-rich fractions have attracted a variety of names but the term 'endosome', which describes the system as a whole, is now becoming the most widely accepted. Unlike other intracellular membranes the limiting membranes of the endosome are cholesterol-rich and contain a relatively narrow range of polypeptides^{16,19}.

In terms of function the most interesting finding has been that endosome membranes contain an ATP-dependent proton pump²⁰. This property accounts most satisfactorily for the now widely confirmed observation made originally by Tycho and Maxfield that ligands internalized by receptor-mediated uptake are rapidly exposed to an environment of low pH²¹⁻²³.

A reduction in pH in the range pH 5-6 is of special significance in the processing of internalized ligand-receptor complexes since it can be expected to separate ligands such as LDL and ASGP from their receptors. It can thus explain how ligands destined for the lysosome become detached and shed from their membrane-bound receptors. In a recent double-label immunoelectronmicroscopical study on the ASGP system in the hepatocyte, just this kind of separation of ligand from membrane-bound receptor has been demonstrated²⁴. Many ligands such as apotransferrin, polymeric IgA and maternal IgG in the neonate gut^{5,6,8} remain as stable complexes with their receptors in this lower pH range. They can thus be expected to remain attached to the limiting membrane of the endosome and are in a position to be routed out of the cell surface-lysosome pathway. The contents of the endosome thus seem to be directed towards the lysosome, while proteins attached to membrane-bound receptors are routed elsewhere. This situation has parallels with the phosphomannose receptor mechanism which separates lysosomal enzymes from secretory product in the rough endoplasmic reticulum-Golgi complex pathway²⁵.

Morphological studies identify the endocytic pathway as including a variety of cisternal and vacuolar elements^{1,3,26,27} extending from the cell surface to the lysosomes lying in the juxtanuclear area (see the figure). The vacuoles often contain small internal vesicles and in the juxtanuclear area usually include what morphologists have for a long time called 'multivesicular bodies'.

The cisternal elements are usually tubular and form tortuous arrays in the peripheral cytoplasm and the juxtanuclear area. These cisternae branch repeatedly and are extensively interconnected with the vacuolar elements. Time-course studies using labelled tracers show that the elements in the peripheral cytoplasm are reached within a few minutes while those in the juxtanuclear area become labelled only after longer intervals. Acidification, ligand dissociation and the retrieval of many receptors such as those for transferrin is so rapid that most of it must take place within the peripheral elements.

The role of the juxtanuclear components is more obscure. However, it seems likely that they have a function distinct from that of lysosomes because, in several systems, a temperature-dependent step, operable only at 20°C and above, has been shown to be required for transfer of the ligands and receptors which they contain to the degradative environment of the lysosome²⁸. It is mainly within the juxtanuclear compartment that the limiting membrane boundary of the endosome appears to pinch off inwards to form multivesicular bodies: a process that may allow removal of receptors from the limiting membrane into the lumen of the system²⁷. In this way receptors can join the mainstream of 'content' travelling towards the lysosome. The retrieval of receptors from the juxtanuclear elements to the cell sur-

face has not so far been clearly demonstrated but there are strong suspicions that it is from this compartment that ligand-receptor complexes are transported to cell-surface domains other than that from which they were derived. The juxtanuclear compartment may thus operate as a redistribution centre for transcellular traffic as well as the site of transfer to lysosomes.

One structural aspect of the endosome that remains to be clarified is the extent to which its membrane boundaries are continuous. Two steps in the endosome pathway are strongly temperature sensitive: the plasma membrane-endosome and the endosome-lysosome transfers. Elsewhere, however, extensive connections between cisternal and vacuolar elements, although often difficult to demonstrate,

clearly exist, and it is possible therefore that much of the routing of integral membrane proteins within the system depends on transfers in the plane of the membrane rather than shuttling vesicles. Consideration of this possibility leads to the general question of what drives and directs the movement of integral membrane proteins within membranes and, for the endosome in particular, whether its receptors should be regarded as purposive migrant proteins²⁹, or passive aggregates carried by a directed membrane flow. The recent papers of Bretscher³⁰ and Ekblom³¹, which have given new impetus to the earlier ideas³² of a directed flow of membrane lipid, clearly suggest the latter. □

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Split genetics

Introns in archaeobacteria

from John Rogers

THE discovery of split genes in archaeobacteria¹ gives support to a pair of radical hypotheses about the earliest living organisms, the 'progenotes'. One of these hypotheses is that RNA splicing, far from being a eukaryotic invention, is a relic of the genetic instability of the progenotes. The other is that archaeobacteria represent a separate line of descent from the progenotes, alongside true bacteria (eubacteria) and eukaryotes.

RNA splicing occurs in all eukaryotes from yeast to man, but not in eubacteria such as *Escherichia coli*. Rather than seek a reason why such a disruptive process should have been newly selected in eukaryotes, Doolittle² proposed that it has always been with us. The progenotes, he suggested, used RNA splicing to link together such short meaningful stretches of RNA as their primitive polymerases could create, and the ancestors of eukaryotes retained this capability; it is the eubacteria which have lost it in 'streamlining their genomes'. (This scheme can also explain why eukaryotes lack the elegant bacterial operon system for coordinated gene expression; it is presumably another aspect of streamlined genomes.) RNA splicing does occur in mitochondria and chloroplasts — putative descendants of symbiotic bacteria — but these could arguably have acquired it from the eukaryotic nuclear genome.

The separate hypothesis that 'archaeobacteria' represent a third line of descent from the progenotes was proposed by Woese and Fox^{3,4}. Their RNA sequence analyses assigned these bizarre prokaryotes a taxonomic status equal with that of eukaryotes and eubacteria. The archaeobacteria combine features of both taxa along with other purely archaeobacterial features, and they could be the least evolved of all organisms.

Putting these two hypotheses together, an obvious corollary is that archaeobacteria, despite their small genomes, might retain RNA splicing. Now, Kaine *et al.*¹ have cloned a cluster of tRNA genes from *Sulfolobus solfataricus* — an archaeobacterium from acidic hot springs — and sequenced two of the genes. These are almost certainly genuine tRNA genes, with anticodons corresponding to serine and leucine, since they have all the characteristic features of tRNA sequences, and they are the only examples of these particular sequences in the genome. Both genes have two features previously seen only in eukaryotic tRNA genes: lack of the 3'-terminal CCA sequence (which must be added post-transcriptionally) and the presence of a short intron.

Each intron comes one nucleotide 3' to the anticodon and could base-pair with the anticodon, just as in eukaryotes. However, this base-pairing must be incomplete; instead, in a uniquely archaeobacterial feature, the boundaries of the introns seem to lie in repeated tetranucleotides (GACC) exposed in symmetrically placed loops.

So the admittedly specialized process of tRNA splicing apparently predates the origin of eukaryotes. One question now is whether the same applies to the much more widespread and versatile process of mRNA splicing, which is what the evolutionary problem is really about. □

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1. Anderson, R.G.W., Brown, M.S. & Goldstein, J.L. *Cell* **10**, 351 (1977).
2. Bridges, K., Harford, J., Ashwell, G. & Klausner, R.D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 350 (1982).
3. Steinman, R.M., Mellman, I., Muller, W.A. & Cohn, Z.A. *J. Cell Biol.* **96**, 1 (1983).
4. Bleil, J.D. & Bretscher, M.S. *EMBO J.* **1**, 351 (1982).
5. Daurty-Varsat, A., Ciechanover, A. & Lodish, H.F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2258 (1983).
6. Klausner, R.D. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 2263 (1983).
7. Renston, R.H. *et al.* *Science* **208**, 1276 (1980).
8. Rodewald, R. *J. Cell Biol.* **85**, 18 (1980).
9. Das, M. & Fox, C.F. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2644 (1978).
10. Pearce, B.M.F. & Bretscher, M.S. *A. Rev. Biochem.* **50**, 85 (1981).
11. Bretscher, M.S., Thomson, N. & Pearce, B.M.F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4156 (1980).
12. Carpentier, J.L. *et al.* *J. Cell Biol.* **95**, 73 (1982).
13. Pastan, I.H. & Willingham, M.C. *Science* **214**, 504 (1981).
14. Willingham, M.C. & Pastan, I. *Cell* **21**, 67 (1980).
15. Kahn, M.N., Posner, B., Varma, A.K., Kahn, R.J. & Bergeron, J.J.M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4980 (1981).
16. Debarne, M.T., Evans, W.H., Flint, N. & Regoecci, E. *Nature* **298**, 398, (1982).
17. Kahn, M.N., Posner, B., Kahn, R.J. & Bergeron, J.J.M. *J. biol. Chem.* **257**, 5969 (1982).
18. Merion, M. & Sly, W.S. *J. Cell Biol.* **96**, 644 (1983).
19. Marsh, M. *Cell* **32**, 931 (1983).
20. Galloway, C.J., Dean, G.E., Marsh, M., Rudnick, G. & Mellman, I. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
21. Tycho, B. & Maxfield, F.R. *Cell* **28**, 643 (1982).
22. van Renswoude, J., Bridges, J., Harford, K.R. & Klausner, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6186 (1982).
23. White, J., Matlin, K. & Helenius, A. *J. Cell Biol.* **89**, 674 (1981).
24. Geuze, H.J. *et al.* *Cell* **32**, 277 (1983).
25. Sly, W.S. & Fischer, H.D. *J. Cell Biochem.* **18**, 67 (1982).
26. Wall, D.A., Wilson, G. & Hubbard, A.L. *Cell* **21**, 79 (1980).
27. McKanna, J.A., Haigler, H.T. & Cohen, S. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5689 (1979).
28. Dunn, W.A., Hubbard, A.L. & Aronson, N.N. *J. biol. Chem.* **255**, 5971 (1980).
29. Brown, M.S., Anderson, R.G.W. & Goldstein, J.L. *Cell* **32**, 663 (1983).
30. Bretscher, M.S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 454 (1983).
31. Ekblom, P., Thesleff, I., Lehto, V.P. & Virtanen, I. *Int. J. Cancer* **31**, 111 (1983).
32. Bretscher, M.S. *Nature* **260**, 21 (1976).

1. Kaine, B.P., Gupta, R. & Woese, C.R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3309 (1983).
2. Doolittle, W.F. *Nature* **272**, 581 (1978).
3. Woese, C.R. & Fox, G.E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5088 (1977).
4. Woese, C.R., Magrum, L.J. & Fox, G.E. *J. molec. Evol.* **11**, 245 (1978).

Obituary

Richard Gershon, 1932–1983

from Martin C. Raff

RICHARD GERSHON died on 11 July 1983, less than a year after he was discovered to have lung cancer and arguably at the peak of his career. As Professor of Immunology and Biology, and Director of the Laboratory of Cellular Immunology of the Howard Hughes Medical Institute at Yale Medical School, he had created one of the largest and most productive immunology groups in the world.

He was born in New York City, attended Harvard College and then Yale Medical School. Apart from two years in Japan in 1961–63 and a year in London in 1967–68, he spent all of his scientific career in the Pathology Department at Yale.

He was a remarkably imaginative and far-sighted scientist whose discovery of suppressor T cells in the early 1970s revolutionized our understanding of how immune responses are controlled. In the following few years, he and his colleagues showed that suppressor T cells regulate both T-cell and B-cell responses and are responsible for some forms of immunological tolerance and genetic unresponsiveness to specific antigens. Together with Harvey Cantor and his colleagues, they went on to show that immune responses are regulated by complex circuits of interacting subclasses of suppressor and helper T cells, which interact with each other and with effector cells by recognizing antigen, major histocompatibility complex molecules and molecules encoded by genes linked to immunoglobulin heavy-chain genes.

His most recent findings were as original and provocative as anything that he and his colleagues had produced before — contra-suppressor cells that protect helper T cells from the effects of suppressor cells, T-cell regulation of antigen-presenting cells, a suppressor factor that is assembled from two different polypeptides, each secreted by a different subclass of T cell — to mention just a few examples. As was the case with most of his work when first presented, these recent results are controversial, but given his remarkable track record, one would be foolish to bet against them.

His greatest asset was an uncanny intuition, which allowed him to see the meaning of experiments well before others in the field. It was as if he knew all along how the immune system worked and was only looking for confirmation. His most fundamental insight was to recognize that there is more to immunity than clonal selection: individual lymphocyte clones do not respond autonomously to antigen, but instead are controlled by specialized regulatory T cells. It was this insight that led him to suppressor cells, rather than the other way round.

Because they often challenged existing

immunological dogma or extended it in quieting ways, his experiments and ideas rarely had immediate impact. In fact, it was several years before his discovery of suppressor T cells was generally accepted, and then it was only after his findings had been confirmed in many other laboratories. But by the mid-1970s, suppressor T cells were turning up everywhere and they rapidly became a 'growth industry', which has

continued to grow ever since. In recognition of his contributions to immunology, he was elected to the National Academy of Sciences in 1980 and was awarded the Gairdner Foundation International Award and Thorn Award in 1983.

It is difficult to imagine an immunological world without Richard Gershon energizing it with his warmth, intellect and *joie de vivre*. He was the life of the immunological party and will be sorely missed. □

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High-energy physics

W and Z particles from CERN

from P.I. Kalms

FOLLOWING the discovery¹ and confirmation² of the existence of the charged intermediate vector bosons, W^+ and W^- , earlier this year, the UA1 Collaboration has recently published evidence^{3,4} for the discovery of the neutral partner of the above, the Z^0 particle.

The early results on the W came from several weeks of collider operation in late 1982, and although few events were found, the mass and event rate were, within experimental uncertainties, as predicted by the standard electroweak theory. The latest results, reported at a recent meeting*, have come from running the CERN proton-antiproton collider from April to July 1983, when the luminosity was higher, so that the number of observed collisions was approximately seven times the 1982 value. For the UA1 experiment this resulted in a total of six examples of the Z particle (four decaying into e^+e^- and two into $\mu^+\mu^-$) and 52 $W^\pm$ particles.

W events ($W^\pm \rightarrow e^\pm \nu$) have the following characteristics. A high-momentum electron passing through the central detector is bent only slightly in the 0.7 T magnetic dipole field. Its sign and approximate momentum can be determined from the track curvature. The track points to a particular cell of the electromagnetic calorimeter which displays a shower characteristic of an electron and gives a precise measurement of its energy. The hadron calorimeter cell behind this shows zero or almost zero energy. Furthermore, if the electron emits bremsstrahlung, for example in traversing the vacuum pipe wall, the photon will travel in almost the electron direction and its energy will be included in the calorimeter signal, which therefore gives a true measurement of the original electron energy.

The presence of the neutrino is found by

constructing a vector sum for the energy flow into all cells of the various calorimeters. The calorimeters cover the complete solid angle down to within 0.2° from the colliding beams. The only losses come from particles remaining within the vacuum pipe and from neutrinos. Muons are detected by chambers outside the calorimeters and thus their momenta can be added to the energy balance. In normal events, which are not expected to involve high-energy neutrinos, the components of energy flow transverse to the colliding beams balance well. The longitudinal component is affected by the particles flowing down the vacuum pipe and is of more limited use. A significant transverse energy imbalance is therefore characteristic of a high-energy neutrino.

The measurement of the electron energy and direction, and the transverse energy of the neutrino leads to the determination of the transverse momentum and 'transverse mass' of each W particle. The distribution of these quantities over a number of W events leads to the actual mass of the W. The transverse momentum distribution of W particles has been determined and is consistent with the production calculations which use quantum chromodynamics (QCD) with emission of hard and soft gluons.

One of the interesting features of W physics at a proton-antiproton collider is that the charge of the W labels the way in which it is produced. On the assumption that valence quarks dominate, a W^+ will be produced from an up quark in the proton and a down antiquark in the antiproton. It has therefore been possible to extract information on the distribution within the proton of those up and down quarks which are sampled by W production.

The angular distribution of the leptons from W decay shows the parity-violating asymmetry characteristic of the weak interaction, that is the positrons from W^+

*The International Europhysics Conference on High-Energy Physics was held at Brighton on 20–27 July.

decay go preferentially in the direction of travel of the antiprotons. The distributions have been found to be consistent with the expected vector minus axial vector form of the weak interaction.

Although, as predicted, Z events are rarer than W events by a factor of about ten, their identification and mass determination are more straightforward, because the angles and energies of both outgoing particles are measured directly. For the $Z \rightarrow \mu^+ \mu^-$ events, the momenta of the particles are measured by the magnetic bending both in the central detector and in the return yoke of the magnet. A more accurate mass determination can be made for the $Z \rightarrow e^+ e^-$ events. The resolution of the electromagnetic calorimeter is numerically about 15 per cent per $\sqrt{E} + 1.5$ per cent where the energy deposited is E GeV. Hence a pair of electrons depositing a total of around 90 GeV should give a mass precision of ± 3.0 GeV. Four such events would reduce this error to ± 1.5 GeV.

But to obtain good absolute mass values for the W and Z particles, an absolute calibration and careful monitoring of the calorimeters is required. Electrons from Z decays and most of those from W decays are measured in large electromagnetic cells called gondolas. Two such gondolas have been placed in a test beam and signals measured when they are struck in various positions and at various angles by high-energy electrons, whose momentum is absolutely determined by measurement of the field integral in a beam magnet. The gondolas are then irradiated with a powerful collimated cobalt-60 γ -ray source and their photomultiplier currents recorded. The same source is then used to produce a detailed map of calorimeter response for all gondolas and other electromagnetic calorimeters. This can only be carried out when the central detector is removed, that is before and after collider runs. During runs, daily monitoring is achieved by shining a measured amount of laser light into the optical system of the calorimeter cells.

A number of other checks were carried out, and it was shown how various small corrections led to the result. The masses presented at the meeting were $m_W = 80.9$ GeV and $m_Z = 95.1$ GeV. In each case there is a statistical uncertainty of ± 1.5 per cent, and a scale uncertainty of ± 3.0 per cent. The scale uncertainty, which is likely to be reduced in the future, would affect the masses of the W and Z particles in similar ways. The values are in good agreement with the predictions of the standard electroweak theory, and the experiment is regarded as a triumph for the CERN laboratory and the collaborating institutes.

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Gene regulation

Enhancer elements activated by steroid hormones?

from Malcolm Parker

STEROID hormones modulate the transcription of specific genes in target cells by the interaction of the steroid-receptor complex directly with specific DNA sequences adjacent to the promoter of responsive genes. Evidence is now emerging that the properties of a glucocorticoid-responsive region in mouse mammary tumour virus (MMTV) resemble those of viral DNA sequence elements known as enhancers¹ that stimulate gene transcription. Since cellular homologues of these viral sequences are required for the activation of immunoglobulin gene expression², it seems that enhancer elements may be essential for tissue-specific expression of a variety of eukaryotic genes.

It is well established that all classes of steroid hormone bind to receptors in target cells to form a steroid-receptor complex which is translocated into the cell nucleus. By means of this cellular mechanism, glucocorticoids stimulate the expression of MMTV DNA in a variety of cell lines. Several laboratories have demonstrated preferential binding of the glucocorticoid-receptor complex to certain cloned MMTV DNA fragments including part of the long terminal repeat containing the viral promoter. In this issue of *Nature*, Beato and his colleagues³ have mapped more precisely the binding sites in the long terminal repeat by using nuclease protection experiments. They find that when glucocorticoid-receptor complex is bound to the DNA, two regions, 72–124 and 163–192 base pairs (bp) upstream from the start of transcription, are protected against DNase I digestion. These two regions therefore seem to be binding sites for the complex. Although the two regions have some DNA sequence homologies, they do not seem to share extensive DNA sequence homology with sequences flanking several other glucocorticoid-responsive genes.

Evidence that such hormone-binding sites operate *in vivo* comes from gene transfer experiments since MMTV DNA can be accurately expressed in heterologous cells and its expression can be stimulated by glucocorticoids. To identify the sequences necessary for hormonal control of transcription, chimaeric genes have been constructed from the MMTV long terminal repeat and various marker genes and this enabled Hynes, Groner and their colleagues⁴ to show that a region in the terminal repeat approximately 220 bp of DNA upstream from the start of transcription is sufficient for full marker sensitivity to glucocorticoids. It thus seems that the glucocorticoid-receptor complex binds directly to DNA sequences adjacent to the

promoter of MMTV and thereby stimulates transcription.

Analogous experiments have been carried out with the chicken egg-white genes, whose expression responds to several classes of steroid hormone. In nuclease protection experiments, Schrader and his colleagues⁵ have shown that the progesterone-receptor complex binds preferentially to a region 150–190 bp upstream from the start of transcription of the ovalbumin gene. However, as with glucocorticoid binding to MMTV, there seem to be multiple hormone-binding sites both upstream of and within the gene^{6,7}. When chimaeric genes containing DNA fragments adjacent to the ovalbumin gene are introduced into oviduct cells, progesterone stimulates expression of the marker gene and the responsive region has now been mapped to a site 95–220 bp upstream from the start of transcription⁸. Similar results have also been obtained by Renkawitz and Schütz and their colleagues for the chicken lysozyme gene^{9,10}. They found that both progesterone and dexamethasone stimulated the expression of a chimaeric gene which had been microinjected into oviduct cells. Both hormone-responsive regions mapped to a region 140–220 bp upstream from the start of transcription. These authors had previously noted a region of partial homology 140 bp upstream in several of the egg-white genes but its role in hormonal control of transcription is unclear because loss of hormone sensitivity of the lysozyme gene occurred even when this region was retained in the chimaeric gene.

Two preliminary observations suggest that the glucocorticoid-responsive region associated with the promoter of MMTV may act as an enhancer to stimulate transcription. First, Hynes *et al.*⁴ have shown that the region is activated by the glucocorticoid receptor even when it is some 500 bp from a promoter, stimulating the transcription of a distal as well as a proximal promoter. Second, in one clone tested, Chandler *et al.*¹¹ have shown that the region can function in either orientation. Enhancer sequences have been identified in other viruses and can stimulate the transcription of heterologous cloned genes as well as viral genes. They act in *cis* over several kilobases, irrespective of their position and orientation. The precise DNA sequence requirement for enhancer activity is unclear but a 'core' sequence 5' GTGG $\uparrow$ $\uparrow$ G 3' is present in several viral enhancers and is essential for activity in simian virus 40. Interestingly, partially homologous regions (for example GTGGTTTC and

1. Arnison, G. *et al.* *Phys. Lett.* **122B**, 103 (1983).
2. Banner, M. *et al.* *Phys. Lett.* **122B**, 476 (1983).
3. Arnison, G. *et al.* *Phys. Lett.* **126B**, 398 (1983).
4. See *Nature* **303**, 473 (1983).

TTGGTTTG) exist in the glucocorticoid-responsive region of MMTV between the two hormone-binding sites.

The obvious question is whether MMTV contains an enhancer whose only difference from those of other viral enhancers is that it requires a steroid hormone for activation or, alternatively, do all classes of steroid hormone modulate gene transcription by activating enhancer sequences? Moreover, in MMTV, although most effort has been devoted to characterizing the glucocorticoid-responsive region associated with the promoter, it is clear that specific hormone binding occurs elsewhere in the viral genome. In view of the location of an enhancer downstream of the immunoglobulin promoter^{2,12-16}, it will be intriguing to discover whether MMTV contains additional enhancers which are activated by glucocorticoid. If enhancer elements play a part in the mechanism whereby all steroid hormones act, then specificity of response to different hormones must reside in the DNA sequence of the enhancer. However, there are genes such as the egg-white and $\alpha 2\mu$ globulin genes whose expression is stimulated by more than one hormone and yet in the case of the lysozyme gene the same region of DNA appears to confer sensitivity to both

progesterone and glucocorticoid. Presumably, hormones must bind to distinct sequences in this region, resulting in activation of one and the same enhancer sequence. □

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1. Khoury, G. & Gruss, P. *Cell* **33**, 313 (1983).
2. Boss, M.A. *Nature* **303**, 281 (1983).
3. Scheideret, C., Geisse, S., Westphal, H.M. & Beato, M. *Nature* **304**, 749 (1983).
4. Hynes, N. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3637 (1983).
5. Compton, J.G., Schrader, W.T. & O'Malley, B.W. *J. Steroid Biochem.* (in the press).
6. Mulvihill, E.R., LePennec, J.P. & Chambon, P. *Cell* **24**, 621 (1982).
7. Compton, J.G., Schrader, W.T. & O'Malley, B.W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6 (1983).
8. Dean, D.C., Knoll, B.J., Riser, M.E. & O'Malley, B.W. *J. Steroid Biochem.* (in the press).
9. Renkawitz, R. et al. *Cell* **31**, 167 (1982).
10. Renkawitz, R., Danesch, U., Matthias, P. & Schütz, G. *J. Steroid Biochem.* (in the press).
11. Chandler, V.L., Maler, B.A. & Yamamoto, K.R. *Cell* **33**, 489 (1983).
12. Gilles, S.D., Morrison, S.L., Oi, V.T. & Tonegawa, S. *Cell* **33**, 717 (1983).
13. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729 (1983).
14. Queen, C. & Baltimore, D. *Cell* **33**, 741 (1983).
15. Emorine, L., Kuehl, M., Weir, L., Leder, P. & Max, E.E. *Nature* **304**, 447 (1983).
16. Neuberger, M.S. *EMBO J.* **2**, 1373 (1983).

Ecology

Evolution of linear habitats in Britain

from Peter D. Moore

IF there is one feature that distinguishes a human hand in the construction of a landscape it is linearity. Linearity is generally displeasing to those who, with blinkered optimism, still like to think of nature as being free from human influence, but the impact of linear features upon the natural world is not always harmful from the point of view of biological diversity¹.

There are cases, of course, where the construction of such features as a road or railway across a habitat can be regarded as harmful because it causes fragmentation and so, by creating barriers to migration, reduces the effective area of that habitat and hence its biological diversity. Usher², for example, claims that the species-area relationship for plants in native reserves in Yorkshire, England, approximates to that expected from classic island biogeographical theory in which $S = cA^z$, where S = species number, A = habitat area and c and z are constants. Reduction in habitat size is thus linked with decreased diversity.

On the other hand, many other considerations must be taken into account in the development of conservation policy, for example, the need for fire breaks in fire-vulnerable habitats where catastrophe could otherwise destroy large areas, and the existence in nature of concentrations of

species in certain peculiar and often small sites. Several commentators have concluded that a number of small sites for conservation can be more valuable than a few large ones^{3,4}.

The linear features themselves may also provide a habitat for plants and animals. Road verges⁵ and railway lines are both recognized as important semi-natural linear habitats. In a recent survey of disused lines in Norfolk, Leaney⁶ found over 500 species of vascular plants in a series of samples totalling 73.5 miles in length, including many rarities for particular parts of the country. Hedges have perhaps received more attention than any other linear habitat⁷ and are generally looked upon favourably by conservationists.

In eastern Britain, particularly in the fens and the Broadland regions, linear systems of dykes or drainage ditches are more frequently used for marking field boundaries and containing stock than are hedges, and these too may represent an important reservoir of aquatic wildlife. The dykes usually have a gradual gradient and hence slowly moving waters⁸, which provides a generally high water table. Often pumps are required to control water movement and, obviously, a lush growth of aquatic plants is frowned upon if they

are to provide an efficient means of land drainage.

Driscoll⁹, in surveying these waterways, has concluded that dykes draining arable land are less rich biologically than those from pasture areas, and the intensity of pastoral management is also related inversely to wildlife potential. In an 8-year survey he documented a decline in aquatic species number of about 17 per cent in areas where a shift from pastoral to arable land had occurred.

Driscoll's work also reveals an overall loss of dyke habitat in recent years, just as has happened with hedges. A detailed survey¹⁰ of dyke patterns in one of his survey sites which has been converted to arable farming showed that, between 1973 and 1981, 33.5 per cent of the dykes had been filled in, and he fears that if this continues, it will have a harmful effect on some of our typically dyke flora, such as the water soldier *Stratiotes aloides*.

Driscoll's maps of past and present dyke patterns also reveal a simplification and fragmentation of the once complex network of waterways, and this leads to the question of whether, and if so how, their loss affects plant and animal dispersal. Are these linear habitats important migration routes for aquatic organisms between larger water bodies?

Similar questions have been asked about our other linear habitats. Railways certainly have provided a means of transport and dispersal for many plant species, the classic example being the Oxford ragwort (*Senecio squalidus*) in the nineteenth century¹¹. The winter-salted road system of Britain is currently providing migration routes for halophytic plants¹². Hedges, on the other hand, do not seem to act as channels for the movement of woodland plants from one relict fragment of forest to another¹³.

The dykes have not yet been examined from this point of view. But if they do prove to have been valuable arteries of dispersal for both plants and animals, it makes their loss doubly regrettable. □

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1. Yapp, W.B. *Biol. Conserv.* **5**, 45 (1973).
2. Usher, M.B.J. *appl. Ecol.* **16**, 213 (1979).
3. Gilpin, M.E. & Diamond, J.M. *Nature* **285**, 567 (1980).
4. Higgs, A.J. & Usher, M.B. *Nature* **285**, 567 (1980).
5. Way, J.M. (ed.) *Road Verges: their Function and Management* (Nature Conservancy, London, 1969).
6. Leaney, R. *Trans. Norfolk. Norwich nat. Soc.* **26**, 112 (1983).
7. Pollard, E., Hooper, M.D. & Moore, N.W. *Hedges* (Collins, London, 1974).
8. Haslam, S.M. *River Plants* (Cambridge University Press, 1978).
9. Driscoll, R.J. *Watsonia* **14**, 276 (1983).
10. Driscoll, R.J. *Trans. Norfolk. Norwich nat. Soc.* **26**, 170 (1983).
11. Kent, D.H. *Proc. bot. Soc. Br. Is.* **5**, 210 (1964).
12. Moore, P.D. *Nature* **297**, 537 (1982).
13. Helliwell, D.R. *Biol. Conserv.* **7**, 61 (1975).

Helioseismology

Oscillations as a probe of the Sun's interior

from Douglas Gough

THE primordial helium abundance, the solar oblateness and its implications concerning General Relativity, the missing neutrinos and the ubiquitous 160-minute period were but a few of the issues pervading a recent conference on helioseismology*. About 80 scientists were present, from most of the major institutions in Europe and the USA in which studies of solar oscillations are being actively pursued.

Solar oscillations can be considered as a superposition of many standing waves, or normal modes. Each is specified by three integers: the order n , the degree l and the azimuthal order m . One can assign to each mode a characteristic wave-number vector, whose vertical and horizontal components are measured by n and l . The component of the horizontal wave number around the equator is determined by m . There are two main classes of modes of immediate interest: acoustic or p modes, for which pressure fluctuations provide the dominant restoring force; and internal gravity or g modes, whose restoring force is predominantly buoyancy. The oscillation frequencies depend on averages of the interior properties of the Sun, with weights that differ from mode to mode. The hope is that a knowledge of a sufficient variety of frequencies will permit us to put stringent bounds on our estimates of conditions inside the Sun.

High-degree p modes propagate nearly horizontally, and are trapped in the outer layers of the Sun. Duvall's wave-guide law¹ is a property of pure acoustic waves uncontaminated by buoyancy. Had the convection zone not been adiabatically stratified the law would not have been satisfied. Thus from a knowledge of the depth of penetration of the modes, we have our first direct estimate, about 1.0×10^5 km, of a lower bound to the depth of the convection zone.

Low-degree p modes penetrate to the core of the Sun. Their frequencies depend on the sound travel time from centre to surface, and on the curvature of the sound speed in the core of the Sun. Observations were reviewed by E. Fossat (University of Nice) and G. Isaak (University of Birmingham), and R. Stebbins (Sacramento Peak Observatory) reported his data acquired recently from the South Pole. R. Yerle and J. Latour (Observatoires du Pic-du-Midi et de Toulouse) discussed limb observations and how they can be interpreted. Fitting theory to the observations selects a theoretical model of the Sun with an initial helium abundance of ~ 25 per cent

by mass. The result is nicely consistent with the high-degree p -mode data, and with current cosmological thinking². However, it is in severe conflict with the measured neutrino flux. The sound speed in the core is roughly consistent with the usual presumption that no substantial mixing of the products of the nuclear reactions with their surroundings has taken place. The models do not fit the data precisely however, so it is not impossible that something is seriously wrong (M. Gabriel, University of Liège; E. Rhodes Jr, University of Southern California; H. Shibahashi, Joint Institute for Laboratory Astrophysics, Boulder). There is evidence from the low-degree data that the problem is outside the Sun's energy-generating core, and J. Christensen-Dalsgaard (High Altitude Observatory, Boulder) remarked that the recent measurements by Duvall and Harvey³ of modes of intermediate degree indicate the difficulties are quite near the surface.

Measurements of possible g -mode frequencies were discussed by P. Delache (Observatoire de Nice), C. Fröhlich (Meteorologisches Observatorium, Davos), V. Kotov (Krymskaya Astrofizicheskaya Observatoria, Nauchny), P. Scherrer (Stanford University) and H. van der Raay (University of Birmingham). Because there is essentially no spatial resolution in the observations, mode identification must be accomplished by fitting the pattern of frequencies with theory. There is still some ambiguity in the procedure, because the frequencies are densely distributed and may be influenced substantially, and to an uncertain extent, by rotation and possibly magnetic fields. Delache and Scherrer reported periods of the form $T_0 (n + \frac{1}{2}l - \epsilon) / [(l+1)]^{-1/2}$ for modes with $l=1$ and 2 and with $n \geq 6$, in accord with high-order asymptotic theory. The value of ϵ was found to be 0.26, close to the 0.25 predicted for the case when the local buoyancy frequency N has a continuous first derivative at the base of the convection zone. The discontinuous gradient of N occurring in standard solar models leads one to expect $\epsilon = 5/12$. Thus perhaps we have the first evidence for mixing between the convection zone and the radiative region beneath on a time scale short enough to influence the thermal stratification. Such mixing is certainly pertinent to understanding the low photospheric lithium abundance. Christensen-Dalsgaard pointed out that the presence of the term $\frac{1}{2}l$ in the factor $n + \frac{1}{2}l - \epsilon$ indicated that the Sun does not have a convective core, in agreement with the evidence from the low-degree p modes

and with stellar evolution theory.

There is some uncertainty in the value of T_0 . Delache and Scherrer claim that the Stanford data yield $T_0 = 38.6$ min, and Fröhlich was able to fit very low frequencies in the SMM-ACRIM irradiance data⁴ to the same value. On the other hand, van der Raay found $T_0 = 41.2$ min. Both these values are somewhat greater than the value of ~ 35 min predicted by the standard solar models that best fit the p -mode data, but G. Berthomieu and J. Provost (Observatoire de Nice) reported that the discrepancy could be removed by assuming a moderate amount of mixing between core and envelope, as Schatzmann and Maeder⁵ have advocated for reducing the neutrino flux. The amount of mixing required, however, is inadequate to resolve the neutrino problem. Moreover it aggravates somewhat the low-degree p -mode discrepancy, especially if van der Raay's value for T_0 is used.

If the Sun were spherically symmetrical, the horizontal variation of the oscillation eigenfunctions would be proportional to a spherical harmonic of degree l and order m , and the eigenfrequencies would be independent of m . Rotation splits this degeneracy, but by whole-disc observations only $l+1$ of the $2l+1$ values of m should be detectable. Thus a stir was caused when Claverie *et al.*⁶ presented evidence for having detected three modes with $l=1$ and five with $l=2$. This could arise if the Sun were not axisymmetric, and Isaak⁹ has suggested the cause of the asymmetry to be an intense internal magnetic field, as Dicke⁸ also advocates. Several theorists have investigated this idea, and at the conference W. Dziembowski (Centrum Astronomiczne im M. Kopernika, Warsaw) reviewed the state of play. He and P. Goode (University of Arizona) presented results for both rotational and magnetic splitting, and announced the rather surprising conclusion that for high-order p modes of low degree the effect of centrifugal distortion exceeded that of advection and Coriolis forces. D. Gough and P. Taylor (University of Cambridge) estimated the centrifugal term to be smaller. According to Goode, the non-uniformity it confers on the splitting seems too great to reconcile with the p -mode identifications claimed previously by H. Hill, R. Bos and himself⁹, so the data may need reinterpretation. These p modes are the modes that had forced estimates of the Sun's angular velocity to rise rapidly through the convection zone, to where its contribution to the quadrupole moment J_2 of the external gravitational potential is greatest¹⁰. Indeed F. Hill (Sacramento Peak Observatory) presented evidence that the Sun's angular velocity decreases with depth immediately beneath the photosphere. Thus the basis for the claim by H. Hill *et al.* for a value of J_2 that, together with the planetary ranging data, contradicts General Relativity seems to have been weakened.

*A conference entitled 'Oscillations as a probe of the Sun's interior' was held at the University of Catania on 20-24 June 1983.

It was emphasized that the influence of a magnetic field that is not symmetric about the axis of rotation is to split the modes of given n and l into $(2l+1)(l+1)$ components, $(l+1)^2$ of which should be visible to whole-disc observations. So the question now is why did Claverie *et al.* see so few? It is possible to choose fields for which frequencies accidentally coincide, or such that some mode amplitudes are very low, as indeed Dicke¹¹ has already argued, but it is not unlikely that that situation would change with time. Therefore we eagerly await the analysis of more recent data.

It should also be borne in mind that it is not only a magnetic field that can lead to severe asymmetry. F. Hill discussed how giant convective cells can perturb the dispersion relation for high-degree p modes, and how one can invert the analysis to deduce from observed perturbations the structure of the giant cells. These cells might also moderate the mode amplitudes, and thus induce anisotropy in the oscillatory power such as was reported by J. Kuhn (Princeton University). Their influence on low-degree modes has yet to be assessed. The result could be an alignment of the modes, which would be manifest on Earth as an amplitude variation in the oscillations caused by the rotation of the wave pattern.

Evidence for the splitting of g modes was reported by Delache, Fröhlich and Scherrer. The power spectrum appears to show only the $l+1$ visible components that one would expect from pure rotation. Participants at the conference were warned, however, that the interpretation of the data is still uncertain, even though the Stanford and SMM results separately implied the same mean rotation period for the Sun's radiative interior: around 9 days. This period is longer than the value I. Roxburgh (Queen Mary College, London) predicted on the assumption that the Sun was everywhere marginally stable to local instabilities, but Roxburgh conceded that by changing initial conditions he could accommodate it. Interestingly, if the radiative interior were to rotate uniformly with this period and the convection zone were to rotate with the photosphere, then $J_2 = 1.5 \times 10^{-6}$, in precise agreement with General Relativity.

There is an increasing interest in new observational tests of theories of the solar cycle. F. Krause (Zentralinstitut für Astrophysik, Potsdam) explained how the torsional oscillation found by Howard and La Bonte¹² is a natural consequence of the α - ω dynamo, and Dziembowski pointed out that it might also be a symptom of Walén's more deep-seated magnetic oscillations. It should be possible to discriminate between these processes by observing 11- or 22-year variations in g -mode splittings.

It is still unclear whether the most important excitation mechanism is the stochastic interaction of turbulence with otherwise stable modes, or whether the modes are self

excited. H. Antia (Tata Institute, Bombay) put the case for the second possibility and N. Dolez (Observatoire du Pic-du-Midi et de Toulouse) set out an ambitious plan to settle the matter by studying with his colleagues the interactions of the modes with their mean environment, with the turbulence and with each other, in a self-consistent way. Van der Raay presented evidence for an increase in oscillatory power after the impact of a comet.

Aside from resonant interactions with p modes, many suggestions for the existence of the 160-min oscillation were put forward. These included its being a nonlinear subharmonic of higher-frequency modes (W. Däppen, University of Cambridge) and a g mode excited by the close passage of another star (A. Kosovichev, Krymskaya Astrofizicheskaya Observatoria) or by the gravitational radiation from binary stars (G. Isaak). It was even suggested that it might be the response to a black hole oscillating about the centre of the Sun and driven by the expansion of its compressed wake arising from the local enhancement of nuclear reactions. Triggered by a remark at IAU Colloquium 66 (ref. 13) on the near commensurability of the rotation periods of the planets with 160 min, Kotov has found that the rotation periods of asteroids have a similar property. He also reported that earthquakes tend to occur at a roughly constant phase with respect to the 160-min oscillation.

The phase relations of the oscillations in the solar atmosphere were reviewed by F.-L. Deubner (University of Würzburg), who suggested that they seemed to imply that the chromosphere-corona transition might lie as high as 3,500 km above the photosphere. J. Staiger (Kippenheuer Institut, Freiburg) reported observations of extremely well defined phase relations between different spectrum lines. These are likely to be of great importance both for diagnosing the structure of the atmosphere and for estimating atmospheric dissipation of the oscillations. That will require a more sophisticated analysis than has so far been accomplished, extending the preliminary results reported by Frandsen (Astronomisk Institut, Aarhus) of a detailed theoretical study of the influence of non-grey radiative transfer on the oscillation eigenfunctions and their damping rates. Staiger also reported finding no vertically propagating waves in the chromosphere with frequencies less than 10 mHz, and G. Poletto (Osservatorio Astrofisico di Arcetri, Florence) estimated the net upward energy flux to be only 1 per cent of the flux in either the upward or downward propagating waves. She concluded that the acoustic energy was insufficient to heat the corona. The heating is produced by the electrical dissipation of Alfvén waves, said L. Campos (Instituto Superior Técnico, Lisbon) as he put forward his theory of the solar atmospheric energy balance.

The 13-day oscillation¹⁴⁻¹⁷ was the topic of considerable discussion. Van der Raay

claimed phase coherence over 5 years and concluded that there must be something in the Sun's core to cause it. B. Anderson (Institut for Teoretisk Astrofysikk, Oslo) argued it was all due to asymmetrical irradiation caused by sunspots, but T. Roca Cortes (Universidad de La Laguna, Tenerife) claimed that the variation of that effect over the last 7 years of the sunspot cycle seemed to be too great to explain the uniformity of the oscillation data. Notwithstanding theoretical estimates to the contrary, Delache argued that the sunspot and plage-induced 13-day variation in the potassium spectrum line used by the Birmingham group might affect the sensitivity of the detector so much as to be responsible for producing the extra l components in the rotationally split low-degree p -mode multiplets discussed earlier, thus bringing the total to the $2l+1$ that Claverie *et al.* have observed. If that were the case, the outer 50 per cent of the Sun by radius would have to be rotating at only the photospheric value, in order to account for the magnitude of the splitting.

Helioseismological studies are still in their infancy, but it is already evident that the powerful diagnostic tool that is being forged can be used elsewhere. Whole-disc measurements can be undertaken for other stars. Shibahashi interpreted the oscillations of an Ap star discovered recently by D. Kurtz (unpublished) as being like the ensemble of solar low-degree p modes, and Fossat reported his possible detection of a 14-min oscillation in α Centauri. It was agreed by the observers that very long periods of continuous observation will be essential to acquire oscillation data with high diagnostic value, and that therefore it would probably be necessary to observe from space. A session was devoted to the devising of a plan for a space mission which will probably be submitted to the European Space Agency next year. Irrespective of whether it will be accepted, the tenor of the meeting was clear: asteroseismology will soon be a reality. $\square$

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1. Duvall, T.L. *Nature* **300**, 242 (1982).
2. Silk, J. *Nature* **302**, 382 (1983).
3. Duvall, T.L. & Harry, J. *Nature* **302**, 24 (1983).
4. Willson, R.C. & Hudson, H.S. *Astrophys. J. Lett.* **244**, L185 (1981).
5. Schatzmann & Maeder *Astr. Astrophys.* **96**, 1 (1981).
6. Claverie, A. *et al.* *Nature* **293**, 443 (1981).
7. Isaak, G.R. *Nature* **296**, 130 (1982).
8. Dicke, R.H. *Solar Physics* **78**, 3 (1982).
9. Hill, H.A., Bos, R.J. & Goode, P.R. *Phys. Rev. Lett.* **49**, 1794 (1982).
10. Gough, D.O. *Nature* **298**, 334 (1982).
11. Dicke, R.H. *Nature* **300**, 693 (1982).
12. Howard & La Bonte *Astrophys. J. Lett.* **239**, L33 (1980).
13. IAU Colloquium 66 *Solar Phys.* **82** (1983).
14. Claverie, A. *et al.* *Nature* **299**, 704 (1982).
15. Durrant, C.J. & Schröter, E.H. *Nature* **301**, 589 (1983).
16. Nyberg Anderson, B. & Maliby, P. *Nature* **302**, 808 (1983).
17. Dicke, R.H. *Nature* **303**, 292 (1983).

Cosmogonical implications of elemental and isotopic abundances in atmospheres of the giant planets

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Current knowledge of element abundances and isotope ratios in the atmospheres of the major planets is reviewed. The observed enhancement of C/H compared with the solar value favours models for the origin of these bodies that invoke the accretion and degassing of an ice-rock core followed by the accumulation of a solar composition envelope. Titan may represent an example of a core-forming planetesimal. Observations of D/H and other isotope ratios must be accommodated by these models in ways that are not yet completely clear. Some additional tests are suggested.

RECENT observations of the atmospheres of Jupiter and Saturn with the Voyager IR spectrometer (IRIS) permit a more precise derivation of isotopic ratios and abundances of minor constituents than has been possible from previous studies¹⁻⁴. When these new results are combined with ground-based determinations of abundances in the atmospheres of Uranus and Neptune, it is possible to derive some useful constraints on possible modes of formation that have been suggested to explain the origin of the giant planets. Here, we shall first review the two principal theories currently in vogue and then see how the new data can be used to discriminate between them.

Models for planetary formation

In the case of Jupiter and Saturn, one approach has been to assume that these planets were formed by local gravitational condensation of the matter in the primordial nebula. These condensations initially encompassed volumes several thousand times greater than the present radii of the planets, in accord with the low density envisaged for the nebula⁵. Subsequent hydrodynamical collapse, followed by slow contraction in hydrostatic equilibrium led to the present state. Throughout this process the composition remained solar and homogeneous. If this is really how these planets formed, the main components of their atmospheres (H₂, He, H₂O, CH₄, NH₃, Ne . . .) should therefore be in solar proportions.

A second general approach, known as the nucleation model⁶⁻⁸ considers that the primordial nebula was at a sufficiently low temperature in the region of the outer planets to permit formation of grains of refractory materials (silicates, iron . . .) and also of ices of H₂O, NH₃, CH₄, and mixed clathrate hydrates of CH₄ and other highly volatile compounds (N₂, CO, and so on). Accretion processes led to the formation of a core. The exact proportions of the potentially available constituents that would actually make up this core cannot be predicted *a priori*. But the model does predict that when the mass of such a core reaches a critical value, surrounding gaseous envelopes mainly made of the abundant gases still more difficult to condense (H₂, He, Ne . . .) will collapse and become gravitationally attached to the core. In such a case, it is possible that during the formation of the core accretional heating revaporizes the ices, which would have subsequently enriched the gaseous envelopes in H₂O,

NH₃, CH₄, and so on, depending on the composition of the core.

Hubbard and MacFarlane⁹ and Stevenson¹⁰ have discussed the advantages and weaknesses of both scenarios in the framework of theories of the internal structures of the giant planets. Recent models of Jupiter and Saturn^{11,12} and of Uranus and Neptune¹³ favour the existence of massive cores in all these planets. At least for Uranus and Neptune, the models require a large proportion of ices in the composition of the cores, which favours the nucleation model and the assumption that the temperature was low enough to condense a substantial amount of various ices from the gas phase at the distances from the Sun at which these outermost planets formed. A model for the origin of Saturn's satellite, Titan, and its atmosphere that is emerging from the analysis of Voyager and ground-based data also favours the accretion of clathrate hydrates as the initial step^{14,15}. Because of its ability to retain the heavier gases released during its accretion, the volatile and non-volatile inventories on Titan may offer us a good example of the kind of material that was available to form the cores of the larger planets. On the other hand, since Titan most probably formed in the proto-saturnian nebula where the pressure, temperature, and composition may have differed significantly from conditions in the solar nebula, comet nuclei may actually be more representative of the postulated core-forming planetesimals.

Constraints imposed by atmospheric abundances

We can make a direct test of the condensation model by comparing the chemical composition of the atmospheres of the jovian planets with the composition of the Sun. The most recent determinations of elemental abundance ratios for all of these planets are given in Table 1, which also contains the solar values. Information on isotope abundances is given in Table 2. The spread in these numbers requires some discussion.

Before the Voyager IR measurements, a large uncertainty existed in the value of the CH₄ mole fraction in Jupiter and Saturn. Most of the determinations of the CH₄ abundance from ground-based measurements were made in the near IR where the effect of the scattering of sunlight by aerosols makes the interpretation of the results strongly model-dependent. In the middle IR, large uncertainties come from telluric absorption,

Table 1 Element abundance ratios in giant planet atmospheres

	Jupiter/Sun	Saturn/Sun	Uranus/Sun	Neptune/Sun	Sun
C/H	2.32±0.18 (2)*	2.1 ^{+1.6} _{-0.5} (19)*	~20 (37) 10–100 (38) ~4 (39)	~25 (37) ~2 (39)	4.7×10 ⁻⁴ (18)
N/H	1±0.5 (1 bar) (1) ~2 (12 bar) (19, 20)	2.4 (3 bar) (19, 20)	<1 (22)	<1 (22)	9.8×10 ⁻⁵ (18)
O/H	1/30 (4 bar, cloud-free region—see text) (1)				6.8×10 ⁻⁴ (40)
P/H	1±0.3 (1)	3.3±0.5 (19)			2.4×10 ⁻⁷ (40)

Source references are indicated in parentheses.

* Revised from the most recent determinations of the ν_4 band intensity of CH₄ (ref. 46).

Table 2 D/H abundance in giant planet atmospheres

	D/H	Molecule	Ref.
Jupiter	3.2 ^{+0.9} _{-1.25} ×10 ⁻⁵	CH ₃ D	1*
Saturn	2.4 ^{+2.4} _{-1.3} ×10 ⁻⁵	CH ₃ D	19
Uranus	4.8±1.5×10 ⁻⁵ ~2×10 ⁻⁵	HD CH ₃ D	41 42, 43
Neptune	(Not yet measured)		
Interstellar	1.5±0.5 0.1~0.5	D D	44 45

* Revised from the C/H values given in Table 1.

from the difficulty of calibrating spectra properly, and from the uncertainty in the atmospheric temperature profile. Recent investigations indicated values of the CH₄ abundance varying from the solar value to 5 times the solar value^{16,17}. The excellent precision and the high spatial resolution of IR spectra recorded by Voyager (permitting selection of data from areas clear of clouds) lead to the definite conclusion that the carbon in Jupiter is enhanced over the solar value of Lambert¹⁸ by a factor of 2 (ref. 2). Preliminary Voyager results for Saturn also indicate an oversolar C/H ratio in this planet¹⁹.

This is a very important conclusion, because methane is the only minor constituent whose abundance in these atmospheres is free from the effects of condensation (as clouds or hazes) or major chemical reactions with other atmospheric gases. Thus the methane–hydrogen ratio should correspond to a solar value of C/H if Jupiter and Saturn formed by direct condensation with no subsequent accretion of large masses of methane-rich planetesimals. The fact that carbon is enriched is therefore a strong argument against a simple condensation model for these planets.

Voyager 2 has not yet visited Uranus and Neptune, and there is a considerable spread in the C/H determinations for these objects from ground-based observations. Nevertheless, the existing data all require a large enrichment of carbon on both planets compared with the solar value (Table 1). This result is consistent in turn with conclusions reached simply on the basis of the relatively high mean densities of these planets: Uranus and Neptune must be made of matter distinctly deficient in light elements compared with the solar composition¹³.

The determination of the N/H ratio is more difficult, even on Jupiter, for two reasons. First, ammonia condenses in the atmospheres of these planets at temperatures lower than about

145 K so that the abundances measured in the upper tropospheres (where we obtain most of our data) do not provide the bulk composition. Second, even at deeper levels, it is possible that some of the NH₃ has been trapped to form clouds of NH₄SH or of NH₃·H₂O. These clouds could exist on Jupiter at pressures of 3–5 bar. No information at levels deeper than 5 bar can be inferred from visible and IR spectra, but it can be obtained from radio emission in the centimetre range. Marten *et al.*²⁰ have inferred the NH₃ vertical distribution of Jupiter and Saturn from radio measurements. For Jupiter they derive an altitude-dependent distribution of NH₃ in the deep atmosphere and find a significant increase of the NH₃ mixing ratio at 3.5 bar, reaching about twice the solar abundance at 12 bar. This result is confirmed and its accuracy improved by the use of the thermal profile retrieved from Voyager data and the use of the H₂/He ratio deduced from Voyager³ in an adiabatic extrapolation to levels below those actually probed by the Voyager instruments.

Similarly, the use of information on these parameters derived from Voyager data on Saturn permits an improvement in the previous results of Klein *et al.*²¹ and Marten *et al.*²⁰ for this planet. The conclusion is that the N/H ratio on Saturn is at least double the solar ratio at atmospheric levels deeper than about 4 bar (ref. 19). These results would be modified, however, if some additional opacity—for example, from H₂O droplets—occurred at centimetre wavelengths²¹.

The interpretation of the 1–21-cm spectrum of Uranus has led Gulkis *et al.*²² to conclude that NH₃ is depleted compared with the solar value in the deep atmosphere of Uranus. The explanation of such a depletion is not clear. The most plausible interpretations are the chemical removal of NH₃ at the 150 K level by a superabundance of sulphur (forming NH₄SH clouds)²² or solution of NH₃ in an ionic 'ocean' at the level of 2,500 K (ref. 10). However, Atreya and Romani (personal communication) find that NH₃/CH₄ may equal the solar mixing ratio if a different extrapolation of NH₃ vapour pressure over water (aqueous ammonia) is used. Since the mixing ratio of methane is also uncertain, we conclude that it is not possible to determine a value of N/H at present. The same considerations apply to Neptune.

H₂O is detectable in the 5- μ m window of Jupiter²³. From Voyager IRIS spectra, Kunde *et al.*¹ have derived a vertical distribution of H₂O in clear regions of the planet where it is possible to detect radiation emerging from levels well below the visible clouds. At the 4-bar level, the H₂O mixing ratio is still only 1/30 of the solar value, but at this level H₂O may be depleted as a result of dynamical processes as it is in the Earth's atmosphere. If the clear regions in the jovian cloud layer are caused by local subsidence—as seems likely from cloud morphology in those places where it can be studied—then these are just the regions where one would expect a low H₂O mixing ratio as the dry cold air of the upper troposphere moves downward towards warmer levels²³. On the other hand, models for the interior of Jupiter have been proposed in which a large core is enriched in ices, which could lead to a depletion of H₂O in the gaseous envelope¹². The resolution of this controversy requires an accurate determination of O/H below the region where water clouds could form. Such a measurement will be carried out by the Galileo probe in 1988.

Another piece of information comes from the measurement of the mixing ratio of phosphine. On the basis of chemical equilibrium models, PH₃ should not be present in the upper atmospheres of these planets²⁴. Yet it is observed in significant quantities, probably as a result of vertical mixing in the lower troposphere. In view of the expected interaction between PH₃ and H₂O, it is not obvious that PH₃ abundances measured in the upper atmospheres can be used to infer the P/H ratio for these atmospheres. The observational evidence tends to support this concern, since the values of P/H found for the two planets are very different (Table 1). Nevertheless, the observed enrichment of P/H on Saturn compared with the solar value seems hard to explain if the true value is simply solar, since the

chemical reaction with H_2O would tend to reduce the amount of PH_3 actually reaching observable levels in the upper troposphere²⁴.

The set of these results seems to favour the scenario of the nucleation model. There is definitely an enhancement of carbon in all four giant planets. There is a strong indication that Jupiter and Saturn are enriched in nitrogen. In cases where a depletion is observed (O/H in Jupiter, possibly N/H in Uranus and Neptune), there is no proof that it concerns the bulk composition of the planet.

However, Stevenson²⁵ has recently argued that convection was not sufficient to move core constituents upwards into the observable regions of the atmospheres of Jupiter and Saturn. If so, the global enrichment in less volatile elements caused by the formation of a large core should not be noticeable. Stevenson proposed instead that the observed enrichment in methane is the result of an accretion of several Earth masses of icy planetesimals by Jupiter and Saturn after their formation. Alternatively, the atmosphere of gases produced from the ices during the process of accretion of the core may have been able to mix with the collapsing envelopes of solar nebula gas during the capture process: such that, the mixing could have occurred during planetary formation, rather than subsequently.

The apparent enhancement of carbon in the atmospheres of the giant planets seems to require condensation or trapping of CH_4 and/or CO in icy planetesimals, probably in the form of clathrate hydrates (such as $\text{CH}_4 \cdot 7 \text{H}_2\text{O}$)^{14,15,25-27}. But it is not obvious where these planetesimals formed. Temperatures below 40 K are required for clathrate formation at plausible pressures in the solar nebula, and such low temperatures suggest distances well beyond Jupiter²⁸. Alternatively, one could imagine the formation of these planetesimals (or at least their continued growth) in the protoplanetary disks. At the higher pressures in such locales, clathrate formation at higher temperatures is feasible, as has been pointed out for the specific case of Titan^{14,15}. If the planetesimals did form at larger distances and then migrated inward to form the jovian core, they must have been rather large objects to avoid loss of their most volatile constituents in times shorter than the core-forming process. Just how massive will depend on the model for the solar nebula that is used to characterize the temperature and radiation field through which the accreting planetesimals must pass.

Constraints imposed by isotope ratios

As Hubbard and MacFarlane^{9,29} have pointed out, a further clue to the origin of Uranus and Neptune is provided by the ratio D/H in their atmospheres. Both of these planets appear to have large cores with relatively thin gaseous envelopes. Hence the composition of the atmospheres of these bodies should reflect the composition of their cores. In the extreme case, one could assume that the atmospheres are nothing more than the result of degassing by the ices in the core, rather like the situation assumed for the atmosphere of Titan but with the ability to retain hydrogen. One expects the ices in the core to represent low-temperature equilibration of D and H in the outer solar nebula. Hence this same equilibration should leave its mark in the hydrogen found in atmospheric gases.

As an example, for $T < 150 \text{ K}$, one finds³⁰ that the fractionation factor f in the relation

$$[\text{CH}_3\text{D}]/[\text{CH}_4] = 4([\text{D}]/[\text{H}])f \quad (1)$$

is > 10 . In other words, a large enrichment in the deuterium in methane should result. This methane is trapped in the ices that form the core and is subsequently released to the atmosphere, where it can exchange deuterium with the atmospheric hydrogen. Since the methane mixing ratio on these two planets is higher than that for Jupiter and Saturn, one would expect a noticeable enhancement in D/H as well. Hubbard and MacFarlane predict an enhancement of ≥ 5 times the nebular value. As Table 2 indicates, this is not the case on Uranus,

where both HD and CH_3D have been observed. Instead, one finds that D/H on Uranus is essentially the same as is found on Saturn and Jupiter, given the uncertainties in the measurements. Furthermore, the values from HD and CH_3 are most consistent for $f \approx 1$, indicating no detectable fractionation between these species. One might expect f to be greater on Uranus than on the larger, warmer planets. We must therefore conclude that either the exchange reaction proceeded so slowly that no large fractionation could occur at the time the planets formed or that the material in the core is not in communication with the atmosphere. The former argument seems the more compelling; the time required to reach equilibrium in reaction (1) at $T < 400 \text{ K}$ has been calculated by Beer and Taylor³⁰ to be greater than the age of the Universe. One would therefore be forced to invoke catalysis³¹ or various non-equilibrium processes³² on an *ad hoc* basis if in fact a high value of D/H existed.

To place this conclusion in context, note that D/H in terrestrial oceans is 1.5×10^{-4} , whereas on Venus it is ~ 100 times this value, as a result of massive escape of hydrogen³⁴. The enhanced value on Earth may result from equilibration of deuterium between H_2O and H_2 in the inner solar nebula or it may have been set in the meteoroids and/or comets that brought volatiles to the inner planets³⁵. Determination of D/H in a comet would be helpful both for its possible relevance to the ratio in terrestrial waters and for the index it would provide on the low temperature equilibration of these important isotopes in the solar nebula.

Another isotope anomaly has been discovered in the atmosphere of Jupiter, where the evaluation of $^{12}\text{CH}_4/^{13}\text{CH}_4$ from Voyager measurements⁴ indicates that $^{12}\text{C}/^{13}\text{C} = 160 \pm 40$, that is $1.8^{+0.4}_{-0.6}$ times the terrestrial-solar value of 90. If this result is confirmed, a plausible explanation of the variation of the $^{12}\text{C}/^{13}\text{C}$ ratio between the inner part of the Solar System and its periphery must be found. The most familiar physical processes which could have occurred in Jupiter—chemical fractionation or neutron irradiation—would have resulted in a decrease of the $^{12}\text{C}/^{13}\text{C}$ ratio compared with the one in the primitive nebula. That is at odds with the observed value if the solar nebular value is, as generally assumed, equal to the terrestrial ratio. Furthermore, neutron irradiation would have affected the carbon isotopes in the inner planets as well, whereas Venus, Mars, and Earth all exhibit the solar value to within 5–10%. Another possibility is that the jovian value is representative of the primitive nebula while the inner Solar System was enriched in ^{13}C during its formation. The jovian value is not in conflict with estimates of the $^{12}\text{C}/^{13}\text{C}$ ratio at the solar neighbourhood 4,600 Myr ago derived from some models of chemical evolution of galaxies applied to recent nearby interstellar medium measurements⁴. In this case, however, one must explain how the Sun itself can exhibit a much lower value than the one posited for the primordial nebula. Further studies of carbon isotopes in all of the outer planet atmospheres are obviously of high priority.

The only other isotopic information we have is a tentative determination of $^{15}\text{N}/^{14}\text{N} = 0.006 \pm 0.001$ on Jupiter by Tokunaga *et al.*³³. This is 1.6 times the solar ratio, but the authors stress that despite the low value of their quoted error, uncertainties in the model atmosphere used to derive this value allow it to be consistent with the solar ratio. Once again there is a clear need for additional observations.

Conclusions

A large and decisive improvement in the determinations of elemental abundances of helium, deuterium, and carbon has been provided by the Voyager encounters with Jupiter and Saturn. In a previous paper³⁶, we have discussed possible constraints on models of big-bang nucleosynthesis implied by Voyager IRIS measurements of deuterium and helium in the atmosphere of Jupiter. The new values of H/He for both Saturn and Jupiter are important boundary conditions for future models of the interiors of these planets as well¹⁰. Here we have addressed the problem of planet formation using all of the

additional abundance data that are available. While some of these determinations are still less certain than one would like, the following conclusions are consistent with what we know at present.

- (1) The atmospheres of Jupiter and Saturn are enriched in carbon compared with the solar atmosphere by about a factor of 2. The enrichment in Uranus and Neptune seems to be still greater. These results favour inhomogeneous models of formation of the giant planets through accretion of planetesimals from the primordial nebula.
- (2) In such a scenario, the outer planets should also be enriched in other heavy elements. There is an indication that such an enrichment in nitrogen exists in the deep tropospheres of both Jupiter and Saturn, but an unambiguous answer for this element and for oxygen will have to wait *in situ* mass spectrometer measurements made aboard probes sent into the atmospheres of these planets.
- (3) The manner in which such an enrichment of the heavy elements took place is not clear. Titan may offer us an example of the type of object that accreted to form the giant planet cores, if these objects grew in protoplanetary disks. We cannot assess the total volatile inventory Titan contains because we do not know to what extent it has degassed. The icy nuclei of 'new' comets from the Oort Cloud may provide a better model for these planetesimals.
- (4) A rock-ice core of solar composition with the ice consisting entirely of methane or CO clathrate would be able to produce the observed carbon enrichment on Jupiter if $M_{\text{core}} \approx 13M_{\text{Earth}}$. Following this idea, one would assume that the accreting cores developed methane (plus CO, N_2 , ^{36}Ar) atmospheres as they formed, so these gases would easily mix with the later collapsing envelope dominated by H_2 and He. In such a case, one predicts a solar value for Ne/H, but ^{36}Ar should be enhanced. The reason for this difference is that neon will not form a clathrate hydrate at temperatures and pressures in any current models of the solar nebula, whereas the properties of argon and methane are quite similar in this regard²⁰. Hence any neon in the atmosphere will be captured with the hydrogen and helium in cosmic proportions to these two most abundant elements.
- (5) Water might have remained frozen in the core (or recon-

densed) in these conditions, leading to a depletion of H_2O in the planetary tropospheres. Conversely, late accretion of the several Earth masses of icy planetesimals required to enhance the jovian methane abundance would bring an even greater enrichment of water in the outer envelopes²⁵.

(6) As Hubbard and MacFarlane⁹ have stressed, a determination of D/H in the atmosphere of Neptune would help discriminate among various explanations of the absence of deuterium enrichment on Uranus. This observation can be made from Earth. The recent measurement of D/H from CH_3D (Table 2) would seem to rule out the possibility that a systematic error in HD observations is responsible⁹. The abundance of deuterium on Titan does not help us, since this isotope is easily enriched as hydrogen escapes. On the other hand, the value of D/H in comets will furnish a good test of low-temperature fractionation of deuterium in icy bodies in the solar nebula.

(7) Probe missions into the atmospheres of all of these outer planets and Titan can be expected to reveal a wealth of additional information on element abundances and isotope ratios. The CNOS abundances as well as the nature of the compounds these elements form in the atmospheres of Uranus and Neptune will provide further tests of hypotheses for the origin of these atmospheres. Intercomparisons of isotope ratios will enable considerable refinement of present ideas about the homogeneity of the solar nebula as well as better definitions of models for the origins and internal structures of these massive planets and their satellites. As another example, the presence of several per cent of primordial argon on Titan would indicate the importance of clathrate hydrates for the origin of that satellite's atmosphere.

At present, we have the prospect of a probe into Jupiter's atmosphere in 1988 as part of the NASA Galileo Project. This will be preceded by ESA and Soviet missions to Halley's comet in 1986, which will provide another pathway towards knowledge about the nature of volatiles incorporated by ices in the outer Solar System. We can anticipate further progress in determinations of isotope ratios from ground-based and Space Telescope observations, but we will need a family of probes into the atmospheres of Titan, Saturn, Uranus, and Neptune to provide additional constraints on planetary formation.

1. Kunde, V. *et al. Astrophys. J.* **263**, 443–467 (1982).
2. Gautier, D. *et al. Astrophys. J.* **257**, 901–912 (1982).
3. Gautier, D. *et al. J. geophys. Res.* **86**, 8713–8720 (1981).
4. Courtin, R., Gautier, D., Marten, A. & Kunde, V. *Icarus* **53**, 121–132 (1983).
5. Bodenheimer, P. *Icarus* **23**, 319–325 (1974).
6. Perri, F. & Cameron, A. G. W. *Icarus* **22**, 416–425 (1974).
7. Mizuno, H., Nakazawa, K. & Hayashi, C. *Prog. theor. Phys.* **60**, 699–710 (1978).
8. Mizuno, H. *Prog. theor. Phys.* **64**, 544–557 (1980).
9. Hubbard, W. B. & MacFarlane, J. *Icarus* **44**, 676–682 (1980).
10. Stevenson, D. A. *Rev. Earth planet. Sci.* **10**, 257–296 (1982); *Planet. Space Sci.* **30**, 755–764 (1982).
11. Slattery, W. *Icarus* **32**, 58–72 (1977).
12. Hubbard, W. B., MacFarlane, J., Anderson, J. D., Null, G. W. & Biller, E. D. *J. geophys. Res.* **85**, 5909–5916 (1980).
13. Hubbard, W. B. & MacFarlane, J. *J. J. geophys. Res.* **85**, 225–234 (1980).
14. Owen, T. *Planet. Space Sci.* **30**, 833–838 (1982).
15. Strobel, D. F. *Planet. Space Sci.* **30**, 839–848 (1982).
16. Combes, M. & Encrenaz, T. *Icarus* **39**, 1–27 (1979).
17. Wallace, L. & Hunten, D. M. *Rev. Geophys. Space Phys.* **16**, 289–319 (1978).
18. Lambert, D. *Mon. Not. R. astr. Soc.* **182**, 249–272 (1978).
19. Courtin, R. thesis, Univ. Paris VII (1982).
20. Marten, A., Courtin, R., Gautier, D. & Lacombe, A. *Icarus* **41**, 410–422 (1980).
21. Klein, M. J., Jansen, M. A., Gulkis, S. & Olsen, E. T. in *The Saturn System* (eds Hunten, D. & Morrison, D.) 195–216 (NASA Rep. CP 2068, 1978).
22. Gulkis, S., Janssen, M. A. & Olsen, E. T. *Icarus* **34**, 10–19 (1977).
23. Larson, H. P., Fink, U., Treffers, R. R. & Gautier, T. N. *Astrophys. J. Lett.* **197**, L137–L140 (1975).
24. Lewis, J. S. in *Chemical Evolution of the Giant Planets* Chap. 2 (ed. Ponnamperna, C.) 13–26 (Academic, New York, 1976).
25. Stevenson, D. *Proc. Lunar Sci. Conf.* (in the press).
26. Miller, S. L. *Proc. natn. Acad. Sci. U.S.A.* **47**, 1798–1808 (1961).
27. Prentice, A. J. R. Preprint (Jet Propulsion Laboratory Publ., 1983).
28. Cameron, A. G. W. in *Moon and Planets* **18**, 5–40 (1978).
29. MacFarlane, J. J. & Hubbard, W. B. in *Uranus and the Outer Planets* (ed. Hunt, G.) 111–124 (Cambridge University Press, 1982).
30. Beer, R. & Taylor, F. W. *Astrophys. J.* **179**, 309–327 (1973).
31. Beer, R. & Taylor, F. W. *Astrophys. J. Lett.* **182**, L131–L132 (1973).
32. Podolak, M. in *Uranus and the Outer Planets* (ed. Hunt, G.) 93–105 (Cambridge University Press, 1982).
33. Tokunaga, A. T., Knacke, R. F., Ridgway, S. T. & Wallace, L. *Astrophys. J.* **232**, 603–615 (1979).
34. Donahue, T. M., Hoffman, J. H., Hodges, R. R. Jr & Watson, A. J. *Science* **216**, 630 (1982).
35. Anders, E. & Owen, T. *Science* **198**, 453 (1977).
36. Gautier, D. & Owen, T. *Nature* **302**, 215–218 (1983).
37. Lutz, B. L., Owen, T. & Cess, R. D. *Astrophys. J.* **203**, 541–551 (1976).
38. Wallace, L. *Icarus* **43**, 231–259 (1980).
39. Fink, U. & Larson, H. P. *Astrophys. J.* **233**, 1021–1040 (1979).
40. Cameron, A. G. W. in *Essays in Nuclear Astrophysics* (eds Barnes, C. A., Clayton, D. D. & Schramm, D. N.) (Cambridge University Press, in the press).
41. Trafton, L. & Ramsay, D. A. *Icarus* **41**, 423–429 (1980).
42. de Bergh, C., Lutz, B. L., Lockwood, G. W., Owen, T. & Buriez, J. C. *Bull. Am. astr. Soc.* **13**, 703 (1981).
43. Lutz, B. L., de Bergh, C., Maillard, J. P., Owen, T. & Brault, J. *Astrophys. J. Lett.* **248**, L141–L145 (1981).
44. Bruston, P., Audouze, J., Vidal-Madjar, A. & Laurent, C. *Astrophys. J.* **243**, 161–169 (1981).
45. Vidal-Madjar, A. *et al. Astr. Astrophys.* (in the press).
46. Brown, L. *Bull. Am. astr. Soc.* **14**, 733–734 (1982).

Computer synthesis of geomagnetic palaeosecular variations

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Computer models, designed to synthesize palaeosecular variations of the geomagnetic field, cast doubt on some widely accepted palaeomagnetic dogmas. Westward (or eastward) drift cannot be uniquely deduced from the sense of rotation of the geomagnetic vector: the use of unit vectors in statistical analyses leads to systematic errors which render investigation of the global symmetry of the palaeofield more difficult than previously supposed. At least some geomagnetic 'excursions' may be regarded as large-amplitude secular variations.

ALTHOUGH palaeomagnetic directions may be determined for a wide range of rock types, the magnitude of the ancient field can be deduced only for a narrow range of materials, such as pottery and some igneous rocks which have remained unaltered since they cooled down: it is rare to encounter studies in which both ancient directions and magnitudes have been determined on the same material. Thus, the subject of palaeomagnetism has been developed essentially using unit vector statistics as introduced by Fisher¹.

It is demonstrated here that errors result from the use of unit vectors rather than total vectors in calculations of mean directions. The existence of these errors has remained unrecognized over the three decades which have elapsed since quantitative studies in palaeomagnetism began. In geological applications of palaeomagnetism to global and regional scale problems these errors are not important because they are small relative to the scale of tectonic motion, but for the investigation of secular variations of the ancient geomagnetic field or of the pattern of its time averaged spatial variations they should be taken into consideration.

Eccentric dipole modelling

A large part of the historically observed secular variation of the geomagnetic field, as recorded instrumentally through the past few centuries, may be represented by westward drifting non-dipole sources and by non-dipole sources which wax and wane in intensity and it has been found useful to separate the non-dipole field (NDF) into drifting and standing parts^{2,3}. Although there is no unique way of modelling the distribution within the Earth's core of the sources of the field, one class of models which has been developed to describe the field at given epochs consists of sets of radial dipoles located deep in the outer core^{4,5}. Alternatively, 'distributed' sources such as electric current loops located near the core surface⁶ have been invoked. Axially orientated eccentric dipoles have also been used to model secular variations⁷ and sets of unconstrained eccentric dipoles have been used to model the whole field⁸. Thus it is not unrealistic to consider simple forms of such models in attempts to understand the longer term behaviour of the geomagnetic field as recorded archaeomagnetically and palaeomagnetically. However, in all the models discussed here, possible attenuation and distortion of time-varying magnetic fields on transmission through the electrically conducting liquid core has been ignored.

Characteristics of some radial dipole models

Let us first consider a class of models in which sources of fixed intensity are allowed to drift.

(a) **Precession of main dipole.** This may be simulated by allowing its equatorial component (GED) of magnitude m to rotate

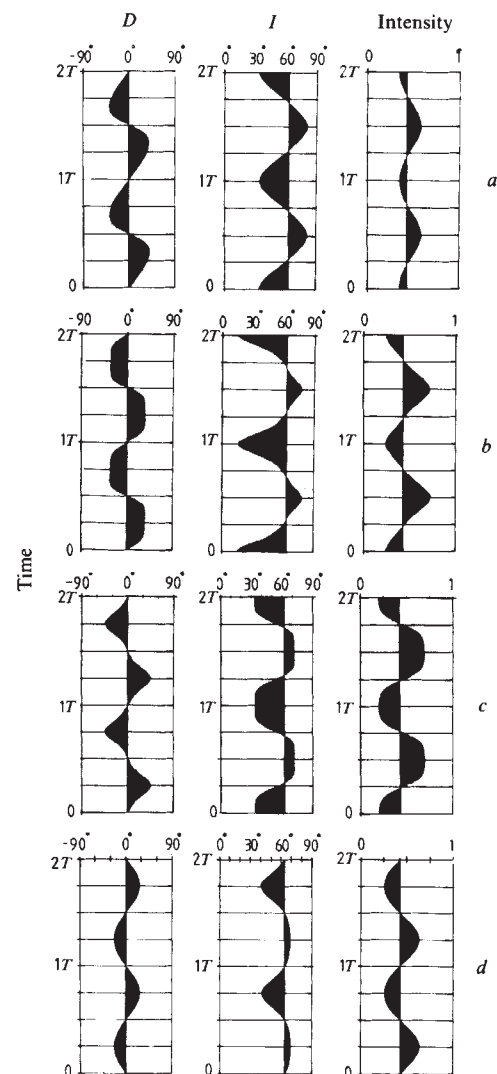


Fig. 1 Declination, inclination and intensity variations produced by: a, a geocentric equatorial dipole (GED) with $m/M = 0.5$; b, a pair of radial dipoles (RD), one pointing up and one pointing down, separated by 180° long., under 45° N lat., located near the bottom of the liquid core at $r = 1,750$ km, $m/M = 0.20$; c, a pair of shallow-seated ($r = 3,400$ km), 'distributed' sources spread around lat. 45° N; d, a single oscillating RD source located at $r = 1,750$ km 30° to the west of the observer, both source and observer being at 45° N lat. Sources a, b and c drift to the west taking time T to lose one revolution relative to the mantle. Source d oscillates with period T . See text for further details.

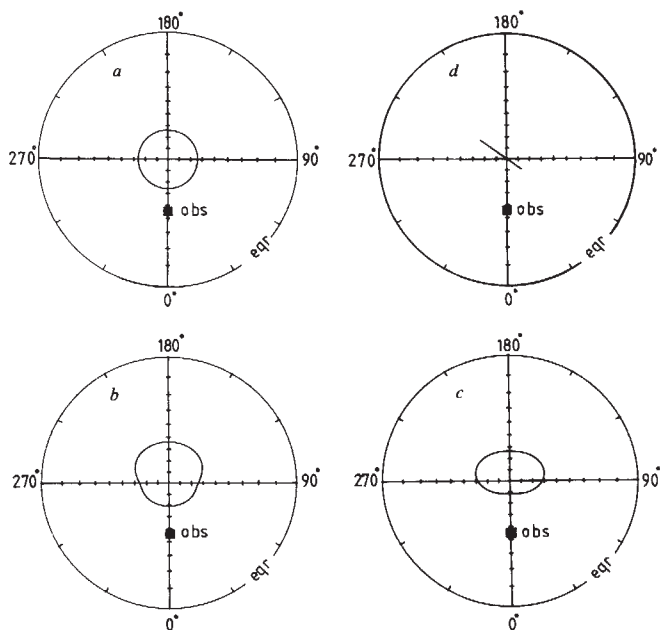


Fig. 2 VGP paths corresponding to models *a*, *b*, *c* and *d* of Fig. 1.

with uniform angular velocity ω about its axial component (GAD) of magnitude M . The resulting time variations of declination, inclination and intensity are shown in Fig. 1a on which the time scale is marked in units of $T (= 2\pi/\omega)$, the period of precession of the resultant inclined dipole. The curves illustrated correspond to the secular variations (SV) which would be recorded by an observer at lat. 45° N for $m/M = 0.50$ for which the geomagnetic axis is inclined to the geographical axis at 26° . In Table 1, the case for $m/M = 0.14$ (geomagnetic axis inclined at 11°) is also included. The SV curves show the following characteristics: the declination and inclination oscillations are out of phase by $T/4$, the declination oscillations are not symmetrical between the zeros and the extreme values and the amplitudes of the inclination minima are larger than those of the maxima. Nevertheless, the corresponding virtual geomagnetic pole (VGP) path is circular and centred on the geographical pole (see Fig. 2a). However, the time averaged inclination, computed over a whole number of rotations of the GED using unit vectors, is shallower than the axial dipole field (ADF) inclination for the site (see Table 1) and therefore the VGP calculated from the time-averaged palaeomagnetic direction will be 'far-sided'. But, when total vectors are used, the true (ADF) average direction is obtained and its VGP will correspond to the geographical pole. Finally, it will be observed that the intensity maxima (as measured from the ADF intensity) are of larger magnitude than the minima and the time averaged

intensity is greater than the ADF intensity (see Table 1). Note that the mean of the components of instantaneous intensity values along the ADF direction will equal the ADF intensity. (b) **Drifting radial dipoles of fixed moment.** The drifting part of the non-dipole field, as computed, for example, by Yukutake and Tachinaka^{2,3}, has an approximate quadrupolar symmetry (see ref. 9) and therefore, for any given epoch, its general form may be represented by four radial dipoles (RD) located at mid-latitudes and separated by 180° long. Two of these RD would be situated under the Northern Hemisphere, one pointing up and the other pointing down, and the other two would be located in the Southern Hemisphere, members of the latter pair being of opposite sign to their companions at the same longitude in the Northern Hemisphere.

The SV in declination, inclination and intensity produced by westward drift of the Northern Hemisphere pair of RDs, each of moment m , as observed at 45° N is illustrated in Fig. 1b. These particular curves are for $m/M = 0.20$ (M being the moment of the GAD) with the RDs located deep in the outer core (at $r = 1,750$ km), like those with which Alldredge and Hurwitz⁴ modelled the geomagnetic field for epochs AD 1945 and 1955. The time scale is again marked in units of the period, T , of one complete revolution of RD drift relative to the surface: for example, if the drift rate were 0.18 deg yr^{-1} , each RD would pass beneath any given meridian periodically at intervals of $T = 2,000$ yr, provided its life time exceeded T , and if the drift rate remained constant.

Note that the shape of declination perturbations is flat-topped and the shapes of the inclination maxima and minima are pointed, the minima being of larger amplitude than the maxima, these unequal magnitudes being attributable to the form of the tangent function. The corresponding VGP path is not symmetrical about the geographical pole (see Fig. 2b). Again, it is necessary to use total vectors rather than unit vectors to calculate the true time averaged field direction (see Table 1) even for axially symmetrical distributions of SV sources. Intensity maxima are of larger magnitude than the minima (as measured from the ADF intensity) and the peaks are 'pointed' in shape. (c) **Drifting current-loop sources of fixed moment.** It is widely accepted, on the basis of skin-depth arguments, that the sources of the non-dipole field SV must be located within the top few hundred kilometres of the outer core. Although such arguments are not strictly valid since they apply to solid conductors while the core is fluid, it is nevertheless instructive to compare the properties of shallow 'distributed' sources with those of deep-seated 'point' sources.

Thus, we may replace each RD by an equivalent electric current-loop which, by virtue of its geometry, would be located at shallower depth closer to the outer-core surface⁶. Such current loops may be modelled by arrays of radial dipoles.

The patterns of SV of declination D , and inclination I , produced by westward drift of two such distributed sources, each simulated by a line of radial dipoles consisting of two blocks, arranged end to end, each extending through 180° of longitude

Table 1 Unit and total vector statistical parameters for synthesized secular variation fields

Model	100 m/M	I_{um}	ΔI	δ_u	δ_t	f_{um}	Δf	s.d.
(a)	50.0	60.2°	-3.2°	20.8°	20.3°	0.456	+0.028	0.035
(b)	14.0	62.8°	-0.6°	8.3°	8.3°	0.433	+0.005	0.036
(b)	± 20.0	57.2°	-6.2°	24.8°	21.9°	0.461	+0.0332	0.160
(c)	0.186	58.7°	-4.7°	17.2°	15.7°	0.445	+0.017	0.171
(d) (0°)	20.0	57.4°	-6.0°	16.0°	13.7°	0.444	+0.016	0.173
(d) (30° W)	20.0	60.5°	-2.9°	13.5°	12.3°	0.438	+0.016	0.137

Models (a)–(d) are described in the text; m , M are the moments of the perturbing radial dipole and of the geocentric axial dipole respectively; axial dipole field parameters, inclination $I_0 = 63.4^\circ$, intensity $f_0 = 0.4278$ Oe; I_{um} and f_{um} are unit vector means of the populations of synthesized field directions; $\Delta I = I_{um} - I_0$ and $\Delta f = f_{um} - f_0$; for all models $I_{tm} = I_0$ and $f_{tm} = f_0$ where subscripts 'tm' signify total vector means; δ_u and δ_t are the angular standard deviations of the populations of synthesized directions using unit and total vectors respectively; s.d. is the corresponding standard deviation of intensities. For model (d) two cases are considered: the RD located directly beneath the observer and 30° to the west of the observer. For the latter model, $D_{um} = 353.9^\circ$ with $\delta_{um} = 13.5^\circ$ and $\delta_{tm} = 12.3^\circ$ with $\Delta D = 6.1^\circ$. For all the other models $D_{um} = D_{tm} = 0$. Units of intensity are oersteds. For model (c), m is moment per deg long.

around a line of latitude (45°N), are shown in Fig. 1c. The shape of the declination anomalies is pointed and the inclination maxima and minima are flat-topped with the minima again having rather larger amplitudes than the maxima. Thus distributed sources can be distinguished from point sources. The corresponding VGP path is shown in Fig. 2c. Again, use of unit vectors rather than total vectors will cause errors in computations of time-averaged field directions (see Table 1). Also the intensity oscillations are flat-topped and the maxima have larger magnitudes than the minima.

(d) **Oscillating or pulsating radial dipoles fixed in position.** In the context of 'standing' sources, secular variations may be introduced, most simply, by regular oscillations, with period T , of the moment m of fixed RD. In such models the sign of the associated non-dipole field (NDF) foci would change periodically. On the other hand, NDF anomalies which simply grow and decay in magnitude, maintaining a positive or negative polarity, may be represented by pulsating sources. The latter may, in turn, be modelled by RDs, the moments of which have an oscillating part m_o and a steady (bias) part m_b with $m_b > m_o$. The basic characteristics of the SV produced by sources which fluctuate irregularly (more realistic geomagnetically) will be similar to those described below for regularly varying sources.

The SV produced by a single, standing oscillating RD source ($m/M = 0.20$) located at $r = 1,750$ km on lat. 45°N , 30°W of an observer located on the surface also at lat. 45°N is illustrated in Fig. 1d. The curves exhibit the following characteristics: the declination anomalies are rounded, the inclination maxima are flat-shaped but the minima are pointed and of larger amplitude

than the maxima. These characteristics would still hold for any single standing source which pulsates, that is which grows and decays maintaining the same polarity, either repeatedly or once only. The VGP path resulting from oscillations of a single source will, therefore, always consist of a straight line as shown in Fig. 2d and one might draw an analogy with plane polarized light. The time averaged directions (D_{um} , I_{um}) for a whole number of complete oscillations computed using unit vectors, will deviate from ADF values: D_{um} will not equal zero and I_{um} will be too shallow. Using total vectors, the mean directions (D_{tm} and I_{tm}) are equal to the ADF values (over a whole number of oscillations). The intensity variations are rounded (Fig. 1d) and the maxima once again are of larger magnitude than the minima.

However, two sources, oscillating with the same period would produce open VGP paths corresponding to circularly or elliptically polarized light. In the more general case, with two sources oscillating with different periods, the VGP paths would look like Lissajous figures. Again, when time averaged directions are computed using unit vectors, I_{um} will be shallower than the ADF value, I_0 , but D_{um} will, in general, not be equal to zero. Using total vectors, the directions, D_{tm} and I_{tm} , averaged over a whole number of oscillations, will correspond to the ADF values (see Table 1).

Interpretation of palaeomagnetic SV curves

The characteristic properties discussed above provide a general guide to the interpretation of palaeomagnetic SV data, especially in terms of the following: (1) interpretation of the

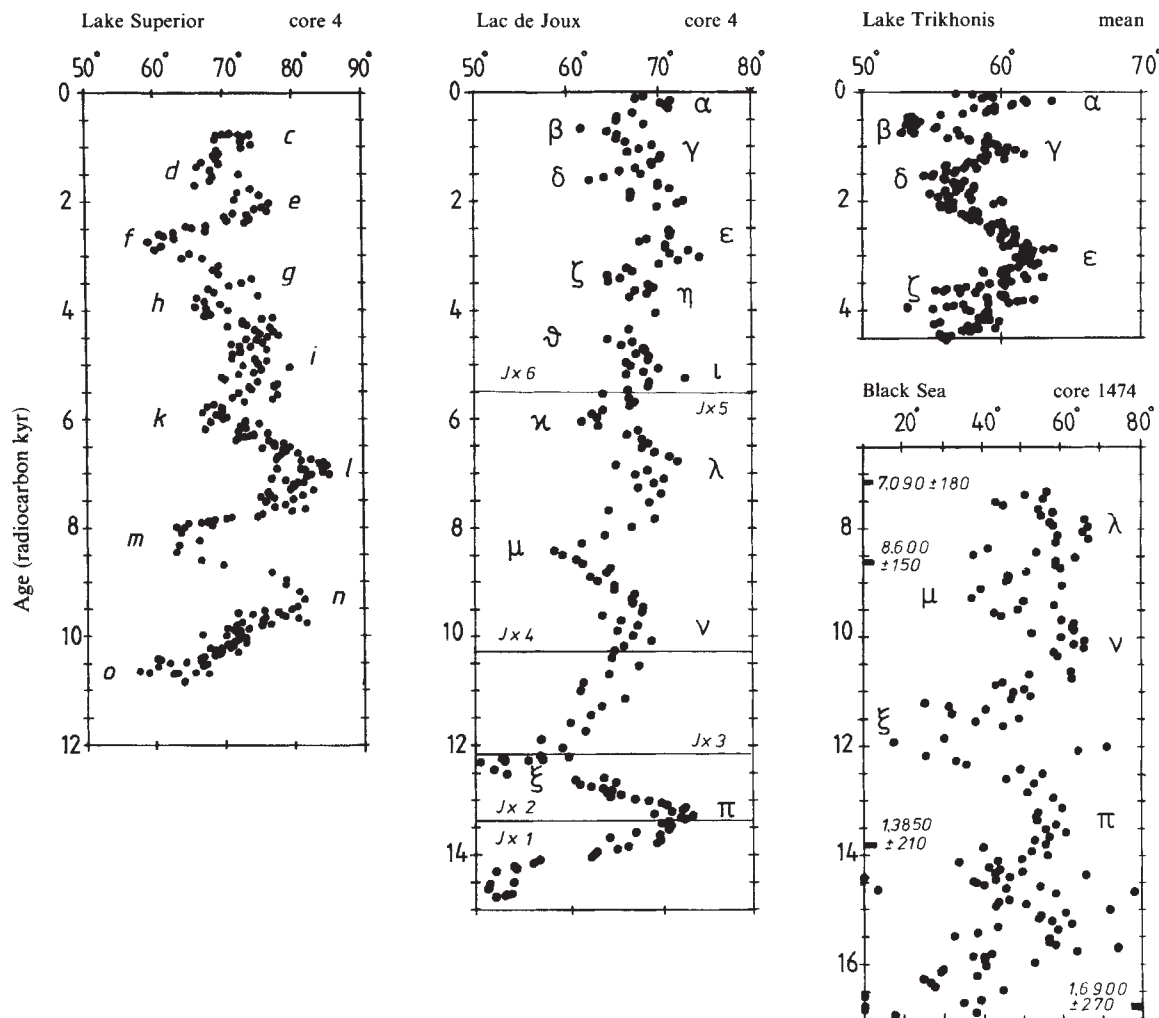


Fig. 3 Palaeoinclination records derived from single sediment cores and exhibiting pointed minima and rounded maxima (as for the model illustrated in Fig. 1b).

sense of rotation of the geomagnetic vector with advancing time in terms of geomagnetic westward (or eastward) drift; (2) distinction between shallow and deep-seated sources; (3) origin of geomagnetic 'excursions'; (4) investigation of the topology of the field on the global scale (for example, long-term persistence of g_2^0 and higher-order terms).

Detection of drift

A word of caution is necessary¹⁰ on the question of the identification of the sense of drift from an inspection of the curvature of VGP plots (which reflect the sense of rotation of the geomagnetic vector) in that, while Runcorn's rule¹¹ for drifting sources, as discussed by Skiles¹², may hold in general, the converse of this rule is certainly not true because the geomagnetic vector can also be caused to rotate by the interference between two or more standing sources which fluctuate in intensity^{10,13}. Thus it is essential that the validity of reports in the existing literature of the occurrence of westward or eastward drift at particular times and at particular places in the past be re-evaluated.

Depth of non-dipole field sources

It should be possible to distinguish shallow, distributed sources from deep-seated, point sources but when we attempt to apply our guidelines to real data (see ref. 13), we find ourselves up against the difficulty that all PSV records contained in sediments are to some extent smoothed due to the processes by which they acquired their remanent magnetization^{14,15}. The effects of smoothing can be simulated in the computer models and it can be shown that the differences between the patterns derived from deep and shallow-seated sources becomes blurred. Similarly, stacking of data to produce 'type-curves' on a regional basis also results in the removal of much of the fine detail recorded in individual cores (see ref. 16 and Chapter 4 of ref. 17).

Nevertheless, it does seem that at least some records of inclination derived from single sediment cores show the characteristic patterns expected of deep seated drifting sources, in that the maxima are rounded and the minima are pointed and of larger magnitude than the maxima (see Fig. 1b). Figure 3 shows some such records from Lake Superior¹⁸, Lac de Joux¹⁹ and the Black Sea²⁰. The sharpness of these minima means that they must have been of short duration: hence one might predict that such features would not have been registered in many palaeomagnetic records, having been smoothed out in the 'lock-in' zone associated with the magnetization process^{14,15}.

Geomagnetic excursions

Abnormally-low inclination perturbation of a main field of normal polarity could be produced by moderately strong deep-seated RD sources which point upwards at mid-latitudes in the Northern Hemisphere (or which point downwards in the Southern Hemisphere). In my computer modelling experiments, I found that changes of sign of inclination could be produced by introducing quite moderate values of m . Particularly sharp inclination perturbations can be synthesized if m is made to fluctuate as it drifts, because the duration of the inclination minimum is then governed by the 'time-constant' characterizing the fluctuations of m rather than that characterizing the rate of drift (see Fig. 5.6 of ref. 21). Such sharp inclination minima could be described as 'geomagnetic excursions'. A given excursion produced in this way would be observed only at different sites within a particular latitude band, if drift were essentially latitudinal, and furthermore only within a limited range of longitudes if the source were a pulsating one. On this basis, we should expect 'excursions' to be of short duration to have been recorded only by sediments which were deposited rapidly. Since rapid sedimentation might be expected to have occurred essentially at times of environmental change (for example, initial stages of deglaciation), it may be no coincidence that many 'excursions' which have been proposed in the literature are derived from palaeomagnetic studies of sedimen-

tary layers deposited at such times (for example, the 'Gothenberg' excursion)²². Yet it is precisely for this reason that their interpretation as records of real geomagnetic phenomena has been questioned on the grounds that the sediments must have been physically disturbed by the high-energy environment of deposition²³. Some evidence concerning the acceptance of 'excursions' as geomagnetic phenomena is reviewed in ref. 24.

Errors arising from use of unit vectors

The models discussed here are symmetrical in the sense that we should expect the secular variations they produce to average to zero over a whole number of cycles. On averaging the directions resulting from running the models under discussion we have seen that the average inclination, I_{um} , calculated using unit vectors is always lower than the axial dipole field value I_0 by some few degrees (see Table 1). However, the average inclination, I_{tm} , calculated by using total vectors (that is by weighting each direction in proportion to its magnitude) is always equal to the ADF value I_0 . For the case of drifting sources, both declination means, D_{um} and D_{tm} , equal zero, but in the case of oscillating RDs, the time averaged declination D_{um} , will not (in general) equal zero though $D_{tm} = 0$ will always. Thus, unit-vector-based analyses of the various data sets synthesized by the above models would lead us to conclude that the steady, background field was not purely that of an axial dipole of moment M , and that persistent non-dipole components existed.

Perhaps the area of palaeomagnetism which is most susceptible to errors introduced by the use of unit vectors is that concerned with global analyses of the time averaged field. Here, palaeomagnetic directions at a given site are averaged over times which are considered to be sufficiently long for SV to be averaged out. The principle conclusion from these studies, as originally pointed out by Wilson^{25,26}, is that the time-averaged field contains a persistent axial quadrupole (g_2^0) term. This result has been confirmed by subsequent work involving different methods of analyses²⁷⁻²⁹. The quadrupole is of the same sign as the main dipole for both normal and reversed configurations of the field and its moment has been estimated at ~5-8% of the main dipole^{29,30}. Its effect is to produce a negative 'inclination anomaly' $\Delta I = \bar{I} - I_0$ at all latitudes in both Northern and Southern Hemispheres: I_0 is the ADF inclination and $\bar{I}$ is the 'time-averaged' value during which secular variations have been assumed to average out. That is to say, $\bar{I}$ is shallower than I_0 in the Northern Hemisphere and steeper than I_0 in the Southern Hemisphere. This is clearly not the same effect as that caused by the errors arising from the use of unit vectors which produce negative ΔI in the Northern Hemisphere (shallow $\bar{I}$) and positive ΔI (shallow $\bar{I}$) in the Southern Hemisphere.

Errors arising from the use of unit vectors would give rise to an apparent g_3^0 term, since most control points are located at latitudes lower than 60° and they would produce a systematic difference between the values of the inclination anomaly as computed for Northern and Southern Hemisphere sites. Such a difference does, in fact, appear in the most recent analyses (see Table 3 of ref. 29) and has been interpreted in terms of an axial octupole of relative magnitude $g_3^0/g_1^0 = +1.7\%$ (normal field) and $+3.4\%$ (reversed field)—see Table 1 of ref. 30.

Thus, the conclusion that the time-averaged field contains a persistent quadrupolar component is not invalidated by the arguments presented here but the evaluation of its magnitude will be in error if the distribution of the input data is biased towards one hemisphere. In fact, it should be well understood that lack of uniformity in the global distribution of control points constitutes one of the most serious sources of error in any spherical harmonic analysis. The apparent existence of a g_3^0 term need not necessarily be attributed to a characteristic property of the geomagnetic field, possibly imposed by the boundary conditions controlling the geomagnetic dynamo process: rather it could be simply due to the use of unit vectors rather than total vectors in computing the time-averaged mean directions on which the global analyses were carried out. Fur-

thermore, I understand that R. Wilson is currently investigating models designed to explain, in terms of errors associated with the use of unit vectors, the easterly bias in declination apparent in analyses of the global palaeofield^{25,26}. Thus, it is clear that

verification of the possible existence of persistent spherical harmonic terms of higher degree and order than g_2^0 must await the establishment of an adequate data bank which includes palaeomagnetic intensities as well as directions.

Received 21 April; accepted 14 June 1983.

1. Fisher, R. A. *Proc. R. Soc. A* **217**, 295 (1963).
2. Yukutake, T. & Tachinaka, H. *Bull. Earthquake Res. Inst., Tokyo* **47**, 65 (1969).
3. Yukutake, T. & Tachinaka, H. *Bull. Earthquake Res. Inst., Tokyo* **46**, 1027 (1968).
4. Alldredge, L. R. & Hurwitz, L. *J. Geophys.* **69**, 2631 (1964).
5. Stearns, C. O. & Alldredge, L. R. *Meth. Comput. Phys.* **13**, 61 (1973).
6. Peddie, N. W. *J. geophys. Res.* **84**, 4517 (1979).
7. Lowes, F. J. & Runcorn, S. K. *Phil. Trans. R. Soc. A* **243**, 525 (1951).
8. Bochev, A. *Pure Appl. Geophys.* **74**, 29 (1969).
9. Creer, K. M. *Nature* **292**, 208 (1981).
10. Creer, K. M. & Tucholka, P. *Phil. Trans. R. Soc. A* **306**, 87 (1982).
11. Runcorn, S. K. *Ann. Geophys.* **15**, 87 (1959).
12. Skiles, D. D. *J. Geomagn. Geoelectr.* **22**, 441 (1970).
13. Creer, K. M. & Tucholka, P. *J. Geophys.* **51**, 188 (1982).
14. Tucker, P. *Geophys. J.R. astr. Soc.* **63**, 149 (1980).
15. Tucker, P. *Earth planet. Sci. Lett.* **56**, 398 (1981).
16. Creer, K. M. & Tucholka, P. *Can. J. Earth Sci.* **19**, 1106 (1982).
17. Creer, K. M., Tucholka, P. & Barton, C. E. (eds) *Geomagnetism of Baked Clays and Recent Sediments* (Elsevier, Amsterdam, in the press).
18. Mothersill, J. S. *Can. J. Earth Sci.* **16**, 1016 (1979).
19. Creer, K. M., Hogg, T. E., Readman, P. W. & Reynaud, C. J. *Geophys.* **48**, 139 (1980).
20. Creer, K. M. *Earth planet. Sci. Lett.* **23**, 34 (1974).
21. Creer, K. M. & Tucholka, P. in *Geomagnetism of Baked Clays and Recent Sediments* (eds Creer, K. M., Tucholka, P. & Barton, C. E.) Chap. 5 (Elsevier, Amsterdam, in the press).
22. Möerner, N. A. & Lanser, J. P. *Nature* **251**, 121 (1974).
23. Thompson, R. & Berglund, B. *Nature* **259**, 490 (1976).
24. Verosub, K. L. & Banerjee, S. K. *Rev. Geophys. Space Phys.* **15**, 145 (1977).
25. Wilson, R. L. *Geophys. J.R. astr. Soc.* **19**, 417 (1970).
26. Wilson, R. L. *Geophys. J.R. astr. Soc.* **28**, 295 (1972).
27. Creer, K. M., Georgi, D. T. & Lowrie, W. *Geophys. J.R. astr. Soc.* **33**, 323 (1973).
28. Georgi, D. T. *Geophys. J.R. astr. Soc.* **39**, 71 (1974).
29. Merrill, R. T. & McElhinny, M. W. *Rev. Geophys. Space Phys.* **15**, 309 (1977).
30. Merrill, R. T., McElhinny, M. W. & Stevenson, D. J. *Phys. Earth planet. Inter.* **20**, 75 (1979).

Priming for and induction of anti-poliovirus neutralizing antibodies by synthetic peptides

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Five peptides containing amino acid sequences from the type 1 poliovirus structural protein VP1 have been synthesized. Each of the peptides was found capable of priming the immune system of rabbits for a long-lasting, virus-neutralizing IgG antibody response following a single inoculation of intact virus. One peptide directly induced the production of neutralizing antibody.

THE mechanisms involved in antibody-dependent poliovirus neutralization have recently been extensively studied (see, for example, refs 1, 2). The virus, a member of Picornaviridae, exists in three stable serotypes, each of which is defined by its inability to be completely neutralized by antibody raised against the other serotypes. Significant neutralizing antibody responses in rabbits are only obtained on inoculation of intact virus. The viral morphogenetic precursors and each of the four detergent-denatured, purified structural proteins (VP1, VP2, VP3, VP4) are generally incapable of eliciting such a response³⁻⁵.

Recent studies have shown, however, that purified VP1 can occasionally give rise to a low level of neutralizing antibody in rabbits⁶. Similarly, in rats, purified VP1 gives rise to a more consistent, but still low level response⁷. Purified VP4 is also occasionally able to induce neutralizing antibodies directed against the intact virion's VP3 (ref. 8), and rabbits inoculated with purified VP1, VP2 or VP3 from formalin-fixed virions produce neutralizing antibodies (van Wezel, personal communication). These responses to the purified structural proteins are, however, generally inconsistent and are probably of too low a level to be protective in susceptible animals. The results obtained with poliovirus are in contrast with those obtained with aphthovirus (foot-and-mouth disease, FMDV), another genus of Picornaviridae. The VP1 of FMDV can elicit neutralizing antibodies^{9,10} and VP1 produced in bacteria from the cloned gene successfully protects susceptible animals from FMDV infection¹¹.

The structures and precise locations of the poliovirion's neutralization epitopes, which are responsible for eliciting neutralizing antibodies, are unknown although they are located on the three structural proteins on the virion's surface (that is,

VP1, VP2 and VP3)¹²⁻¹⁴. Studies with neutralizing monoclonal antibodies have shown that a number of neutralization epitopes are clustered on VP1^{1,15-17}, the predominantly exposed surface protein¹⁴. Consistent expression of these epitopes is lost when the virus is disrupted and its proteins are purified.

The studies reported here describe the selection and synthesis of five peptides composed of virus-specific sequences from regions of VP1. Four of these peptides were able to interact

1 GLY-LEU-GLY-GLN-MET-LEU-GLU-SER-MET-ILE-ASP-ASN-THR-VAL-ARG-GLU-THR-VAL-GLY-ALA-ALA-THR-SER-ARG-ASP-ALA-LEU-
#5

26 PRO-ASN-THR-GLU-ALA-SER-GLY-PRO-THR-HIS-SER-LYS-GLU-ILE-PRO-ALA-LEU-THR-ALA-VAL-GLU-THR-GLY-ALA-THR-ASN-PRO-

55 LEU-VAL-PRO-SER-ASP-THR-VAL-GLN-THR-ARG-HIS-VAL-GLN-HIS-ARG-SER-ARG-GLU-SER-SER-ILE-GLU-SER-PHE-PHE-
#1 #4

82 ALA-ARG-GLY-ALA-CYS-VAL-THR-ILE-MET-THR-VAL-ASP-ASN-PRO-ALA-SER-THR-THR-ASN-LYS-ASP-LYS-LEU-PHE-ALA-VAL-TRP-
#2 #3

109 LYS-ILE-THR-TYR-LYS-ASP-THR-VAL-GLN-LEU-ARG-ARG-LYS-LEU-GLU-PHE-PHE-THR-TYR-SER-ARG-PHE-ASP-MET-GLU-LEU-THR-

136 PHE-VAL-VAL-THR-ALA-ASN-PHE-THR-GLU-THR-ASN-ASN-GLY-HIS-ALA-LEU-ASN-GLN-VAL-TYR-GLN-ILE-MET-TYR-VAL-PRO-PRO-

163 GLY-ALA-PRO-VAL-PRO-GLU-LYS-TRP-ASP-TYR-THR-TRP-GLN-THR-SER-ASN-PRO-SER-ILE-PHE-TYR-THR-TYR-GLY-THR-

190 ALA-PRO-ALA-ARG-ILE-SER-VAL-PRO-TYR-VAL-GLY-ILE-SER-ASN-ALA-TYR-SER-HIS-PHE-TYR-ASP-GLY-PHE-SER-LYS-VAL-PRO-

217 LEU-LYS-ASP-GLN-SER-ALA-ALA-LEU-GLY-ASP-SER-LEU-TYR-GLY-ALA-ALA-SER-LEU-ASN-ASP-PHE-GLY-ILE-LEU-ALA-VAL-ARG-

244 VAL-VAL-ASN-ASP-HIS-ASN-PRO-THR-LYS-VAL-THR-SER-LYS-ILE-ARG-VAL-TYR-LEU-LYS-PRO-LYS-HIS-ILE-ARG-VAL-TRP-CYS-

271 PRO-ARG-PRO-PRO-ARG-ALA-VAL-ALA-TYR-TYR-GLY-PRO-GLY-VAL-ASP-TYR-LYS-ASP-GLY-THR-LEU-THR-PRO-LEU-SER-THR-LYS-

298 ASP-LEU-THR-THR-TYR

Fig. 1 Amino acid sequence of the poliovirus type 1 (Mahoney strain) VP1 structural protein. The sequences contained in the synthetic peptides used for this study are underlined.

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Fig. 2 Amino acid sequences of the five synthetic peptides. Residues derived from the VP1 sequence are underlined (see Fig. 1). Each peptide contained two Gly residues as spacers followed by an amino-terminal Tyr or Cys residue. Peptides 1, 2, 3 and 5 were linked to BSA as carrier via the terminal Tyr. Peptide 4 was linked to KLH via the Cys residue. The -Gly-Gly- spacers were included to provide some distance between the main peptide sequence and the carrier molecule. It was reasoned that this would minimize any effects the carrier might have on the conformational possibilities of the peptide. The penultimate Gly was labelled with tritium to provide a tracer and method of assessing the efficiency of coupling to the carrier. Only in peptides 1, 2 and 5 was an additional carboxy-terminal Gly residue used to anchor the respective peptide to the resin during synthesis. The peptides were synthesized by the solid phase method^{33,34}. The initial amino acid was covalently attached to a *p*-methylbenzhydrylamine resin (US Biochemical Corporation; 96 mM available amine per g resin). The resin was stored in the salt form and was, therefore, deprotonated by the addition of 5.0% diisopropylethylamine (DIEA, Aldrich) in methylene chloride before the introduction of the initial amino acid. The coupling cycle was initiated using equimolar amounts of tBoc AA-OH (Peninsula) and dicyclohexylcarbodiimide (Pierce) in three to fourfold molar excess over available amine. The coupling reactions were allowed to proceed for 1–2 h and subsequently washed with alternating methylene chloride/isopropanol washes. The efficiency of the coupling reaction was assessed by either a picric acid titration³⁵ or a ninhydrin assay³⁶. If the efficiency of the coupling was not $\geq 99\%$, the coupling was repeated, such that each coupling reaction was $\geq 99\%$ efficient. The tBoc group, used to protect the α -amino group of the newly added amino acid, was removed with 50% trifluoroacetic acid (Pierce) in methylene chloride. The peptide-resin was then washed and deprotonated with 5.0% DIEA before proceeding to the next coupling cycle. For Asn and Gln reactions, an equimolar amount of 1-hydroxybenzotriazole in dimethylformamide (Sequantol grade; Pierce) was added to the protected amino acid. Di-tert-butyl-pyrocyanate (US Biochemical Corp) was used to derivatize a ³H-Gly to obtain the tBoc-[³H]Gly-OH (ref. 37). The following side-chain protecting groups were used: *O*-benzyl for Thr, Ser, Asp and Glu; tosyl for Arg; methoxybenzyl for Cys; Cl-Z for Lys; and Cl₂-Z for Tyr. The final peptide-resin was treated with half its weight of anisole and five times its volume of anhydrous hydrogen fluoride (HF; Matheson) and allowed to react for 30 min at 0 °C to affect cleavage of the peptide from the resin. The HF was distilled from the reaction and the mixture washed three times with petroleum ether. The peptide was then extracted from the mixture with 1% acetic acid and purified by ion exchange chromatography and partition chromatography. The purified peptide was coupled to BSA by the methods of Bassiri *et al.*³⁸ and Walter *et al.*³⁹. Approximately 30 mol peptide per mol BSA were linked. The KLH linkage used was a modification of Lui *et al.*⁴⁰ in which the *N*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce) was first conjugated to the KLH, followed by rapid purification on a Sephadex G-25 column. The activated KLH was then cross-linked to the peptide. The final product was purified by dialysis. 1,600 mol peptide per mol KLH were linked.

Peptide 1	H ₂ N-TYR-GLY-GLY- <u>ARG-SER-ARG-SER-GLU-SER-GLY</u> -COOH
Peptide 4	H ₂ N-CYS-GLY-GLY- <u>ARG-SER-ARG-SER-GLU-SER-SER-ILE-GLU-SER-PHE</u> -COOH
Peptide 2	H ₂ N-TYR-GLY-GLY- <u>SER-THR-THR-ASN-LYS-ASP-LYS</u> -GLY-COOH
Peptide 3	H ₂ N-TYR-GLY-GLY- <u>ASP-ASN-PRO-ALA-SER-THR-THR-ASN-LYS-ASP-LYS</u> -COOH
Peptide 5	H ₂ N-TYR-GLY-GLY- <u>ASP-ASN-THR-VAL-ARG-GLU-THR</u> -GLY-COOH

with neutralizing monoclonal antibodies, suggesting that they can form structures similar or identical to the neutralization epitopes of the intact virion. Linked to a carrier protein, each peptide was found capable of priming the immune system of rabbits for the production of a stable, significant, serotype-specific neutralizing antibody response upon a single presentation of intact virus. In addition, one of the carrier-linked peptides was able to induce directly a similar neutralizing antibody response. We suggest that the priming phenomenon of small peptides may lead to a new strategy in vaccination.

Peptide sequence selection

Figure 1 presents the amino acid sequence of the capsid polypeptide VP1 of type 1 (Mahoney strain) poliovirus. The sequence was obtained by deduction from the nucleotide sequence of the viral RNA^{18,19} and by the identification of the terminal amino acid residues of the purified protein^{20–22}. The sequences used in the synthesis of the five peptides (Fig. 1) were chosen on the basis of (1) a hydrophilicity analysis of the protein sequence (according to the method of Hopp and Woods²³ and D. Davison, unpublished) and (2) a sequence variation analysis obtained by comparing the amino acid sequences of the VP1 of the three poliovirus serotypes (A. Nomoto, personal communication).

All five peptides correspond to hydrophilic regions of VP1. It was assumed that such regions are more likely to be exposed on the virion's surface^{24,25}. Peptides 1 and 4 encompassed the area of the highest hydrophilicity in VP1. Peptides 2, 3 and 5 included regions with considerable inter-serotype sequence variability. Such variability may be the result of genetic selection directed by the environmental presence of neutralizing antibodies.

The peptides (Fig. 2) were synthesized, by solid-phase procedures, to include the VP1-specific sequences and a -Gly-Gly-spacer followed by a Tyr or Cys residue at the amino terminus. The latter amino acids were used for linking the peptides to suitable carrier proteins for immunization.

Recognition by neutralizing antibodies

THE synthetic peptides were tested by enzyme-linked immunosorbent assay (ELISA) for their ability to be recognized by neutralizing monoclonal antibodies that had been raised against the intact virion of type 1 (Mahoney strain) poliovirus. Each of these antibodies binds to a different neutralization epitope and none is capable of recognizing epitopes on the intact VP1^{1,15}. The results in Table 1 show that four of the five peptides could, in fact, assume conformations recognizable by the antibodies. However, such conformations appear to be expressed by only a small fraction of the total peptide population as none of the peptides was found to inhibit viral neutralization completely by any of the antibodies (data not shown).

As the synthetic peptides are exact copies of specific sequences from VP1, these data suggest that the isolated VP1 cannot assume a configuration by which these sequences are exposed in the proper conformations to interact with the monoclonal antibodies. Isolated VP1 may either be locked into an unfavourable configuration or may undergo rapid configurational changes none of which can form stable complexes with the antibodies.

The extent of binding of poliovirus-specific amino acid sequences to monoclonal antibodies is size dependent even for small peptides. For example, peptide 1 bound to antibodies of groups A, B, C and E whereas the extended peptide 4 bound only to antibodies of group B. The small peptide 2, on the other hand, was recognized only by antibodies of group F whereas the extended peptide 3 bound to antibodies of groups D and F. The size-dependent change of peptide binding to monoclonal antibodies cannot be explained, but may be due to both the specific amino acid sequence of the peptide and the constraints placed on the expression of the sequence's conformation by the length of the peptide.

The observation that peptides corresponding to sequences of two regions in VP1 interact specifically with all the available neutralizing monoclonal antibodies identifies these sequences as major neutralizing antigenic sites of poliovirus. This does

not mean, however, that poliovirus bears only these two neutralizing sites. On the contrary, the reports that capsid polypeptides other than VP1 can induce neutralizing antibodies (see above) show that these proteins also bear antigenic sites involved in virus neutralization.

Finally, the accuracy of peptide recognition by monoclonal antibody was thoroughly supported by our observation that only peptide 2 binds monoclonal antibody C3 (data not shown), a unique antibody that both neutralizes type 1 poliovirus and immunoprecipitates purified VP1¹⁷. The C3-binding site resides in the region corresponding to peptide 2 as determined using a series of partially deleted VP1 molecules²⁶.

Neutralizing antibody

Peptide induction of neutralizing antibodies has recently been achieved with aphthovirus (FMDV)^{27,28}. It was of interest to determine whether any of the poliovirus-specific peptides, when linked to a carrier protein, would elicit similar responses. Only peptide 3 induced a significant level of neutralizing antibodies in three of four rabbits (Table 2). Studies are in progress to determine the general ability of this peptide to elicit neutralizing antibodies in animals other than rabbits. The data support our conclusion that amino acids 93–103 are part of a major poliovirus antigenic site involved in neutralization. The same site has also been identified in poliovirus type 3 by structural analyses of viral monoclonal antibody non-neutralizable variants¹⁶. The sequence of this site is different for each serotype of poliovirus (A. Nomoto, personal communication).

The failure to elicit neutralizing antibodies with the other four peptides does not mean that these sequences *per se* are inactive. The extension of our studies to different experimental animal systems may yield different results.

Peptide priming

The ability of peptides to interact specifically with neutralizing monoclonal antibodies reflects the peptides' ability to fold into configurations that resemble neutralization epitopes of poliovirus. Even in the absence of a neutralization response it seemed reasonable to assume that these small peptides, when presented to an animal's immune system, would be 'seen' in configurations similar to the virion's surface. We therefore investigated whether the peptides were capable of 'priming' and analysed the sera of rabbits that had been inoculated with the peptides first and subsequently injected with intact virions.

By the end of the peptide inoculation schedule, all peptides tested elicited an IgG immune response as analysed by ELISA. With the exception of antibodies against peptide 3 (in one of two animals, see above), none of the sera was capable of neutralizing or immunoprecipitating virions or purified VP1 (data not shown). A single injection of these rabbits with virions, on the other hand, yielded neutralization sera of high titre. These results are summarized in Tables 3 and 4.

Peptide 1 was capable of priming for a significant, long-lasting neutralizing IgG response against type 1 poliovirus (rabbits A and B). A single virus inoculation of an unprimed rabbit (rabbit D) or of a rabbit inoculated with a peptide unrelated to poliovirus sequences (rabbit E) gave no response. The priming effect was seen even after a single inoculation of carrier-linked peptide (rabbit C). A lower-level neutralizing IgG response against type 2 virus was also, but not always, obtained following inoculation of primed rabbits with type 1 virus.

Such cross-neutralization is similar to that seen in anti-type 1 hyperimmune serum (rabbit F). Furthermore, the anti-type 2 response in hyperimmune serum was enhanced by a single inoculation of peptide. The occasional neutralizing anti-type 3 activity seen in the type 1-inoculated animals was not IgG in nature and gradually disappeared.

Peptide 1 failed to prime for significant neutralizing antibody responses when followed by inoculation with types 2 or 3 virus (rabbits G and H). A good anti-type 2 response was achieved, however, when this virus was inoculated with type 1 virus (rabbit I). The inability of peptide 1 to prime for an anti-type 3 response

was surprising because the sequence of this peptide is completely conserved between types 1 and 3 viruses (ref. 16 and A. Nomoto, personal communication). However, a non-neutralizing anti-type 3 virion IgG response was obtained on type 3 (rabbit H) and even type 1 (rabbit B) virus inoculation of primed rabbits, as the corresponding sera could immunoprecipitate type 3 virions. It seems that this region of VP1 is exposed to the surface in virions of all three types. In types 1 and 2, this sequence assumes the role of a neutralizing antigenic site and, hence, its interaction with antibody inactivates the virion. However, in type 3 virions, binding of this site with antibodies does not interfere with infection.

Each of the other four peptides was also capable of priming the rabbits' immune system for a neutralizing antibody response

Table 1 Recognition of synthetic peptides by neutralizing monoclonal antibodies

Antibody		Peptide				
		1	2	3	4	5
H3	(B)	+	–	–	+	–
ICJ 31-10	(E)	+	–	–	–	–
D3	(A)	+	–	–	–	–
A13	(C)	+	–	–	–	–
ICJ 27	(D)	–	–	+	–	–
1 BM 55-6	(F)	–	+	+	–	–

+, Positive response by ELISA using a 1:10 dilution of monoclonal antibody-containing hybridoma culture supernatant. –, ELISA reaction was not significantly above background control. The ELISA reaction was carried out in a microtitre plate. 50 µg of peptide in phosphate-buffered saline (PBS) was adsorbed onto each well overnight. Unadsorbed sites were blocked with 0.5% gelatin solution in PBS. After extensive washing, the test antibody in PBS was added and incubated at room temperature for 2 h. The wells were washed with 0.05% Tween-20 in PB followed by PBS alone. A 1:250 dilution of alkaline phosphatase-linked goat anti-mouse IgG (Tago) was added to each well and incubated at room temperature for 3 h. The wells were washed as above. 0.1% *p*-nitrophenyl phosphate (Sigma) in 10% diethanolamine, 0.5 mM MgCl₂, pH 9.8, was added to the wells. Colour was allowed to develop for 2 h at 37 °C. Neutralizing monoclonal antibody was raised against type 1 (Mahoney strain) poliovirus^{1,15}. The epitope grouping to which the antibody belongs¹ is given in parentheses.

Table 2 Anti-poliovirus immune response induced by peptide 3

Rabbit	Weeks after final peptide inoculation	Plaque reduction (log ₁₀ PFU ml ⁻¹)			Antiserum (i.p.)*		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
A	1	2.1	<1.0	<1.0	+	–	–
	5	2.0	<1.0	<1.0	+	–	–
B	1	<1.0	<1.0	<1.0	–	–	–
	5	0.9	<1.0	<1.0	+	–	–
C	1	1.0	<1.0	<1.0	+	–	–
	5	1.2	<1.0	<1.0	+	–	–
D	1	1.0	<1.0	<1.0	+	–	–
	5	1.0	<1.0	<1.0	+	–	–

The rabbits were inoculated with 1.0 mg per inoculation of BSA-linked peptide (1:1 with complete Freund's adjuvant (CFA) by the intradermal (i.d.), subcutaneous (s.c.) and intramuscular (i.m.) routes at 0, 4 and 5 weeks, respectively. Plaque titre reduction values were determined by a standard plaque reduction neutralization test. Approximately 7.0–9.0 log₁₀ plaque-forming units (PFU) per ml stock virus were diluted nine times by serial 10-fold dilutions. 0.1 ml of each dilution was incubated with either 0.1 ml PBS or 0.1 ml undiluted test serum for 1 h at room temperature. Each dilution was then used to infect a 35-mm diameter dish containing a confluent HeLa cell monolayer. Developed plaques were counted 48–72 h post-infection. The plaque titre reduction values were calculated by comparing the plaque counts of the saline-treated and serum-treated samples.

* Immunoprecipitation of ³⁵S-methionine-labelled, purified virus by antiserum using *Staphylococcus aureus* protein A. This test measures the presence of an IgG response against the virus³². +, Immunoprecipitation of labelled virus was at least 10-fold above control background value.

Table 3 Priming of anti-poliovirus immune response by peptide 1

Rabbit	Inoculated poliovirus type	Weeks after virus inoculation	Plaque titre reduction (log ₁₀ PFU ml ⁻¹)			Antiserum (i.p.)		
			Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
A	1	1	>8.5	1.4	1.0	+	—	—
		3	>8.5	1.8	<1.0	+	—	—
B	1	1	>8.5	2.3	2.2	+	+	+
		5	>8.5	2.2	<1.0	+	+	+
		9	>8.5	1.8	<1.0	+	+	+
C	1	1	>8.5	2.6	1.7	+	+	—
	(Single peptide inoculation)	5	>8.5	2.1	<1.0	+	+	—
D	1	1	<1.0	<1.0	<1.0	—	—	—
	(No peptide; single virus inoculation)							
E	1	1	<1.0	<1.0	<1.0	—	—	—
	(Control peptide)							
F	1	1	>8.5	3.9	<1.0	+	+	—
	(Hyperimmune)							
	(+ peptide)	1	>8.5	6.7	<1.0	+	+	—
G	2	1	1.5	2.7	<1.0	—	+	—
		5	<1.0	3.0	<1.0	—	+	—
H	3	1	<1.0	<1.0	<1.0	—	—	+
I	1, 2, 3	1	>8.5	>6.7	<1.0	+	+	—
		5	7.5	>6.7	>1.0	+	+	—

Rabbits A, B, G, H and I were inoculated as follows: 1.0 mg per inoculation of BSA-linked peptide 1 (diluted 1:1 with CFA) was inoculated into each rabbit by the i.d., s.c. and i.m. routes at 0, 4 and 5 weeks, respectively. Approximately 5.0 log₁₀ PFU of the appropriate poliovirus type(s) (1:1 with CFA) was inoculated, i.m., 10 days following the final peptide inoculation. The following virus strains were used: type 1 (Mahoney), type 2 (Lansing) and type 3 (Leon). Rabbit E underwent an identical inoculation schedule using a Rous sarcoma virus src sequence-specific decapeptide (linked to KLH as carrier protein) which bore no sequence homology with any poliovirus protein sequence. Rabbit C received only the first i.d. inoculation of linked polio-specific peptide. Four weeks later, this was followed by i.m. inoculation with virus. Rabbit D received only a single i.m. inoculation of 5.0 log₁₀ PFU poliovirus type 1 (1:1 with CFA). Rabbit F was made hyperimmune to type 1 poliovirus by the inoculation of 50 µg of virus (1:1 with CFA) by the i.d., s.c. and i.m. routes at 0, 4 and 5 weeks, respectively. Ten days following the last virus inoculation, the rabbit received, i.m., 1.0 mg BSA-linked peptide (1:1 with CFA).

Table 4 Priming of anti-poliovirus immune response by peptides 2, 3, 4 and 5

Peptide	Rabbit	Inoculated poliovirus type	Weeks after virus inoculation	Plaque titre reduction (log ₁₀ PFU ml ⁻¹)			Antiserum (i.p.)		
				Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
2	A	1	1	>8.5	3.6	3.3	+	—	—
	B	1, 2, 3	1	5.5	3.2	1.4	+	+	—
			5	6.3	2.7	<1.0	+	+	—
3	B	1, 2, 3	1	7.8	1.9	<1.0	+	—	—
			5	8.0	<1.0	<1.0	+	—	—
			5	6.7	<1.0	<1.0	+	—	—
4	A	1	1	6.7	<1.0	<1.0	+	—	—
			5	6.2	<1.0	<1.0	+	—	—
	B	1, 2, 3	1	5.8	1.3	<1.0	+	—	—
5	A	1	1	>8.5	1.7	<1.0	+	—	—
			5	>8.5	<1.0	<1.0	+	—	—
	B	1, 2, 3	1	6.7	1.8	<1.0	+	—	—
			5	6.5	<1.0	<1.0	+	—	—

All rabbits were inoculated with 1.0 mg per inoculation of carrier protein-linked peptide (diluted 1:1 with CFA) by the i.d., s.c. and i.m. routes at 0, 4 and 5 weeks, respectively. Approximately 5.0 log₁₀ PFU of the appropriate poliovirus type(s) (1:1 with CFA) was inoculated, i.m., 10 days following the final peptide inoculation. The data for rabbit A of peptide 3 are shown in Table 2.

(Table 4). Rabbits primed with peptide 2 developed neutralizing IgG antibodies against type 1 virus and, to a lesser extent, type 2 virus. Rabbits primed with peptides 3, 4 and 5 were more specific in their response, giving rise to stable neutralizing IgG antibodies only against type 1 virus.

The degree and specificity of the neutralizing response seemed to be influenced by the peptide used for priming. Peptides 1 and 2 primed the immune system for a higher level of antibody production while peptides 3, 4 and 5 primed for a more serotype-specific response. The former response may be due to the ability of the short peptides, 1 and 2, to fold into a larger number of neutralization epitope-bearing conformations. The increased specificity of the remaining peptides probably reflects the greater number of amino acid differences among the serotypes in those regions of VP1 from which these peptide sequences were derived (A. Nomoto, personal communication).

Finally, the following observations support the conclusion that priming is specific to the peptides and not due simply to the bovine serum albumin (BSA) carrier protein which has been subjected to the coupling procedure. First, peptide 4 successfully primed the immune system for an antiviral response (Table 4) while being coupled to keyhole limpet haemocyanin (KLH) as carrier (Fig. 2 legend). In addition, a BSA-linked peptide containing an amino acid sequence from the viral VP3 structural protein failed to induce neutralizing antibodies or to prime the immune system of inoculated animals (unpublished observations).

Conclusions

Synthetic peptides corresponding in sequence to a segment of the tobacco mosaic virus capsid protein were used previously to produce neutralizing antibodies against tobacco mosaic virus^{29,30}, yet the potential of this strategy has been recognized

only recently (reviewed in refs 24, 25). In spite of the knowledge of the complete amino acid sequences of many viral surface proteins, it has been difficult to elicit neutralizing antibodies with carrier-linked synthetic peptides. So far, only one system has been reported in which such immunization offered definite protection against subsequent challenge with virulent virus^{27,28}.

The potential of a synthetic peptide as a vaccine-suitable antigen has usually been assessed by the peptide's ability to induce neutralizing antibody. Here we have shown that such potential can also be tested with neutralizing monoclonal antibodies in a simple binding assay. More importantly, however, we have presented evidence that selected peptides, although themselves unable to elicit neutralizing antibodies, can specifically and efficiently prime an immune system. As a single presentation with homologous virus resulted in a stable, high-titre neutralizing antibody response, peptide priming offers a new strategy in vaccination.

The priming with poliovirus-related sequences is specific in that peptides containing a serotype-specific amino acid sequence will generally prime for an immune response against that serotype of poliovirus alone. This specificity is an important property of the peptides because wide-spectrum priming of the immune system by a single peptide is undesirable. The strategy of priming can also be used to identify neutralization epitopes, particularly if a large panel of monoclonal antibodies is not

available. In fact, it may be very difficult to generate neutralizing monoclonal antibodies against all neutralizing epitopes because some epitopes, on the intact virion, are more immunopotent than others. This seems to be the case with the region in VP1 which corresponds to peptide 5.

Whether peptide-mediated priming can protect a mammal against 'naturally' acquired infection with virulent virus is now being tested. It may be necessary to follow priming with the presentation of a natural antigen such as the inactivated or oral poliovirus vaccines. It is intriguing to speculate, however, that in infections characterized by a long incubation time, such as viral hepatitis, priming may offer sufficient protection to prevent clinical disease.

A preliminary report of this work has been presented elsewhere³¹.

We thank Bruce Erickson and Bruce Merrifield for instructing us in peptide synthesis, Radu Crainic and Florian Horodniceanu for supplying monoclonal antibodies, Tom Hopp and Bert Semler for helpful discussions, David Gates and Joan Brugge for providing a control peptide, Jean Leibowitz, Lee Ann Austin, Marlies Schmidt and Anita Lewis for laboratory assistance, and Susan Studier for the preparation of the manuscript. This work was supported by PHS grants AI-15122 and CA-28146. E.A.E. is the recipient of a PHS Postdoctoral Fellowship AI-06485.

Received 14 April; accepted 28 June 1983.

1. Emini, E. A., Kao, S., Lewis, A. J. & Wimmer, E. *J. Virol.* **46**, 466-474 (1983).
2. Icenogle, J., Shiwen, H., Duke, G., Rueckert, R. & Anderegg, J. *Virology* (in the press).
3. Dernick, R. *Dev. Biol. Stand.* **47**, 319-333 (1981).
4. Melen, R. H., Rowlands, D. J. & Brown, F. J. *gen. Virol.* **45**, 761-763 (1979).
5. Rueckert, R. R. in *Comprehensive Virology* Vol. 6 (eds Fraenkel-Conrat, H. & Wagner, R. R.) 131-213 (Plenum, New York, 1976).
6. Blondel, B., Crainic, R. & Horodniceanu, F. *C. r. heb. Séanc. Acad. Sci., Paris* **294**, 91-94 (1982).
7. Chow, M. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7518-7521 (1982).
8. Emini, E. A., Dörner, A. J., Dörner, L. F., Jameson, B. A. & Wimmer, E. *Virology* **124**, 144-151 (1983).
9. Laporte, J., Grosclaude, J., Vantchem, J., Bernard, S. & Rouze, P. *C. r. heb. Séanc. Acad. Sci., Paris* **276**, 3399-3401 (1973).
10. Bachrach, H. L., Moore, D. M., McKercher, P. D. & Polatnick, J. J. *Immun.* **115**, 1636-1641 (1975).
11. Kleid, D. G. *et al. Science* **214**, 1125-1129 (1981).
12. Beneke, T. W., Habermehl, K. O., Diefenthal, W. & Buchholz, M. *J. gen. Virol.* **34**, 387-390 (1977).
13. Lonberg-Holm, K. & Butterworth, B. E. *Virology* **71**, 207-216 (1976).
14. Wetz, K. & Habermehl, K. O. *J. gen. Virol.* **44**, 525-534 (1979).
15. Emini, E. A., Jameson, B. A., Lewis, A. J., Larsen, G. R. & Wimmer, E. *J. Virol.* **43**, 997-1005 (1982).
16. Minor, P. D. *et al. Nature* **301**, 674-679 (1983).
17. Blondel, B., Akacem, O., Crainic, R., Coullin, P. & Horodniceanu, F. *Virology* **126**, 707-710 (1983).
18. Kitamura, N. *et al. Nature* **291**, 547-553 (1981).
19. Racaniello, V. R. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4887-4891 (1981).

20. Emini, E. A., Elzinga, M. & Wimmer, E. *J. Virol.* **42**, 194-199 (1982).
21. Larsen, G. R., Anderson, C. W., Dörner, A. J., Semler, B. L. & Wimmer, E. *J. Virol.* **41**, 340-344 (1982).
22. Semler, B. L., Hanecak, R., Anderson, C. W. & Wimmer, E. *Virology* **114**, 589-594 (1981).
23. Hopp, T. P. & Woods, K. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3824-3828 (1981).
24. Walter, G. & Doolittle, R. F. in *Genetic Engineering* Vol. 5 (eds Setlow, J. K. & Hollaender, A.) 61-91 (Plenum, New York, 1983).
25. Sutcliffe, J. G., Shinnick, T. M., Green, N. & Lerner, R. A. *Science* **219**, 660-666 (1983).
26. Van der Werf, S. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
27. Bittle, J. L. *et al. Nature* **298**, 30-33 (1982).
28. Pfaff, E., Musgay, M., Böhm, H. O., Schultz, G. E. & Schaller, H. *EMBO J.* **1**, 869-874 (1982).
29. Anderer, F. A. & Schlumberger, H. D. *Biochim. biophys. Acta* **97**, 503-509 (1965).
30. Anderer, F. A. & Schlumberger, H. D. *Biochim. biophys. Acta* **115**, 222-224 (1966).
31. Wimmer, E., Emini, E. A. & Jameson, B. A. *Rev. infect. Dis.* (in the press).
32. Ey, P. L., Prowse, S. J. & Jenkin, C. R. *Immunochemistry* **15**, 429-436 (1978).
33. Erickson, B. W. & Merrifield, R. B. in *The Proteins* (eds Neurath, H. & Hill, R. L.) 255-527 (Academic, New York 1976).
34. Barany, G. & Merrifield, R. B. in *The Peptides* (eds Gross, E. & Meienhofer, J.) 1-284 (Academic, New York, 1979).
35. Gisin, B. F. *Anal. chim. Acta* **58**, 248-249 (1972).
36. Sarin, V. K., Kent, S. B. H., Tam, J. P. & Merrifield, R. B. *Analyt. Biochem.* **117**, 147-157 (1981).
37. Pozdnev, V. F. *Chem. nat. Compds* **10**, 782-784 (1974).
38. Bassiri, R., Dvorak, J. & Utiger, R. in *Methods of Hormone Radioimmunoassay* (eds Jaffe, B. & Behrman, H. R.) 45-55 (Academic, New York, 1979).
39. Walter, G., Scheidtmann, K. H., Carbone, A., Laudano, A. P. & Doolittle, R. F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5197-5200 (1980).
40. Lui, F., Zinnecker, M., Hamaoka, T. & Katz, D. H. *Biochemistry* **18**, 690-697 (1979).

Bacteriophage λ protein *cII* binds promoters on the opposite face of the DNA helix from RNA polymerase

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*The bacteriophage λ transcriptional activator protein, *cII*, coordinately regulates transcription from two phage promoters that control lysogenic development. We demonstrate that *cII* is a DNA binding protein that selectively interacts with a repeat sequence in the -35 region of the promoter. Furthermore, *cII* is shown to bind mainly one face of the DNA helix and to make its contacts primarily in the major groove of the DNA. RNA polymerase sees this same region from the opposite side and sandwiches the DNA helix between itself and *cII*.*

THE *cII* protein of bacteriophage λ is a positive regulator of RNA transcription^{1,2}. This regulatory protein coordinately activates transcription from two different promoter signals, *P_{RE}*

and *P_L*, positioned far apart on the phage genome (Fig. 1; see refs 3, 4 for review). These promoters control the expression of the two phage functions, repressor (*cI*) and integrase (*int*),

required for lysogenic development. Previous work by this laboratory demonstrated directly that *cII* alone was both necessary and sufficient to activate P_{RE} and P_I transcription⁵. Moreover, high level expression of *cII* protein was obtained in *Escherichia coli*, thereby allowing its purification and detailed physical and biochemical characterization⁶.

The *cII* gene and its ribosome binding site have been shown to overlap structurally with *cII*-dependent P_{RE} promoter and this region of overlap has been extensively characterized^{4,7,8}. Twenty-seven different mutations were genetically and structurally defined within the overlapping 36-base pair (bp) region⁷. This small region contains information important to P_{RE} promoter function, *cII* gene translation and *cII* gene function^{4,7-10}.

Here, we examine the direct interaction of *cII* protein and RNA polymerase with the P_{RE} and P_I promoter signals. DNase protection experiments demonstrate that *cII* interacts specifically in the -35 regions of both promoter signals. Chemical probe experiments define more precisely the interaction of *cII* and RNA polymerase with these regions of the template. In addition, 16 different point mutations in this region are used to delineate specific base pairs which are important for *cII* and/or RNA polymerase interaction.

Transcriptional activation

Using a purified *in vitro* transcription system, we demonstrated previously that P_{RE} and P_I promoter function was dependent on *cII* protein⁵. To study this *cII* dependence in more detail, we monitored and compared transcriptional activation of P_{RE} and P_I in response to increasing concentrations of *cII* protein. The titration curves are shown in Fig. 2a. The results indicate that in optimized transcription conditions (saturating *cII* levels and in the presence of excess RNA polymerase) the P_{RE} promoter is about four times more efficient than P_I . This difference is similar to the relative efficiencies exhibited by these two signals *in vivo* during a normal phage infection^{11,12}. However, in marked contrast to the differences observed in promoter strengths, P_{RE} and P_I show essentially identical activation responses to *cII* protein. That is, both promoters start to function and reach their respective maxima at the same *cII* concentrations. The half-maximal levels of activation for both signals were found to be approximately 2×10^{-8} M.

These experiments suggest that *cII* interacts and functions almost identically at both the P_{RE} and P_I signals. Apparently, the fourfold difference observed in promoter efficiency is due to differences in the ability of RNA polymerase (rather than *cII*) to interact at these sites. Promoter competition experiments lead to the same conclusions. P_{RE} and P_I were made to compete for limiting amounts of *cII* protein within the same transcription reaction. No competition was observed. Both promoters start to function at the same *cII* concentration and exhibit the same relative activation responses as was observed in separate reactions (not shown). Thus, P_{RE} and P_I interact equally with *cII* and their different efficiencies reflect differences in RNA polymerase activity.

In light of these results, it is important to note that the P_{RE} and P_I signals have markedly different -10 region sequences and use radically different nucleotides for initiation (ATP for P_{RE} and UTP for P_I)^{6,11-15}. In contrast, the -35 regions of P_{RE} and P_I exhibit extensive sequence homology; 11 identical base pairs out of 14 are positioned the same distance from their respective transcription start sites. We demonstrate below that *cII* protein interacts specifically in this region of sequence homology and exhibits the same affinity for both the P_{RE} and P_I signals. We suggest that the different efficiencies of these two promoters result largely from differences in the ability of RNA polymerase to recognize their non-homologous -10 region recognition and start-site sequences.

cII binds the -35 region

DNase protection (footprinting) experiments have been used to demonstrate and study the selective interaction of a variety

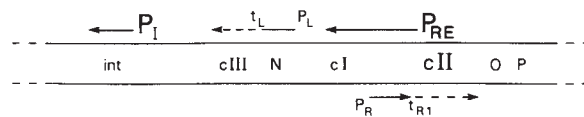


Fig. 1 Partial genetic map of bacteriophage λ . The positions of the *cII* activated promoters, P_{RE} and P_I , are indicated as well as the directions of transcription (arrows). The major leftward promoter (P_L) and the major rightward promoter (P_R) are also shown. The *cII* gene is transcribed from P_R and is positioned just beyond the first rightward termination signal (t_{R1})⁸. The N-terminal coding region and ribosome recognition signal of the *cII* gene overlap with the P_{RE} promoter⁷. P_{RE} controls expression of the phage repressor (*cI*) whereas P_I controls expression of integrase (*int*).

of proteins with DNA¹⁶⁻²¹. We have used the same procedures to examine *cII* and RNA polymerase interaction with both the P_{RE} and P_I promoter signals. Both DNase I and neocarcinostatin (NCS) nucleases were used to probe the protein-DNA interaction. DNA fragments which span either the P_{RE} or P_I regions were labelled at one end and then subjected to partial endonucleolytic digestion either in the absence or in the presence of increasing amounts of *cII* protein. The fragments generated were separated by electrophoresis on standard DNA sequencing polyacrylamide gels. DNA sequencing reactions carried out on the same end-labelled fragments served as position markers. Figure 3 shows typical results from these experiments.

The data demonstrate that *cII* protein binds to DNA and specifically protects the -35 region sequences of both the P_{RE} and P_I promoters. The protected region encompasses some 20-24 bp and centres precisely in the -35 region sequence homologies shared by the P_{RE} and P_I promoters. The actual binding site, however, is probably smaller than 20 bp. *cII* binding is likely to cause some nonspecific steric hindrance of DNase cleavage and certain nucleotide positions cannot be monitored due to their insensitivity to cleavage. This tends to enlarge the protected region artificially. Moreover, the fact that the -35 region homology shared by P_{RE} and P_I extends only 14 bp and encompasses all the known -35 region promoter down mutations^{7,8} suggests that the *cII* binding site may only encompass about 14 bp. Although direct evidence is still lacking, we presume that a single 44,000 molecular weight *cII* tetramer (the native form of the protein⁶) binds to this region of DNA.

We also used the footprinting procedure to measure the affinity of *cII* for DNA. Protection experiments were carried out in the presence of increasing concentrations of *cII* protein and the concentration of *cII* required to obtain half-maximal protection of the P_{RE} and P_I binding sites was determined. The results (Fig. 2b) indicate that *cII* has the same affinity for both the P_{RE} and P_I sites; the apparent binding constant, K_a , was 2×10^{-7} M. Similar measurements have been made for other DNA binding proteins using essentially the same procedure^{20,21}. For example, the cyclic AMP-dependent transcriptional activator protein, CRP, was shown to bind selectively to a site on the plasmid pBR322 with an affinity similar to that shown here for *cII* protein¹⁹. In contrast, most repressor proteins tend to show much stronger affinities for their binding sites. Presumably, the differences in DNA affinity exhibited by repressor and activator molecules reflect the different functions carried out by these two types of regulatory protein when interacting with DNA.

Footprinting experiments were also used to examine the interaction of RNA polymerase, both alone and in the presence of *cII*, with the P_{RE} and P_I signals. Alone, RNA polymerase showed little, if any, interaction at either promoter. However, at high RNA polymerase concentrations, we did note a small but reproducible alteration in the DNase I digestion pattern within the -10 region sequence at P_{RE} (Fig. 4). A similar result was seen at the P_I site (not shown). The importance of this observation is unclear, as other methods of detection (for

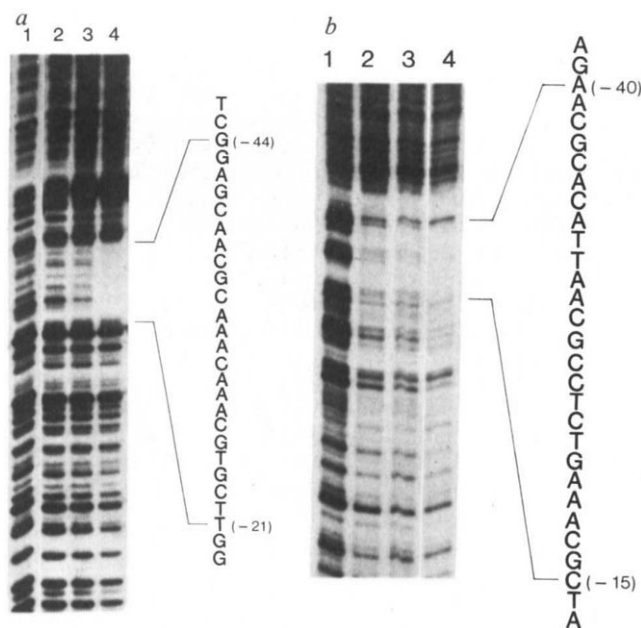
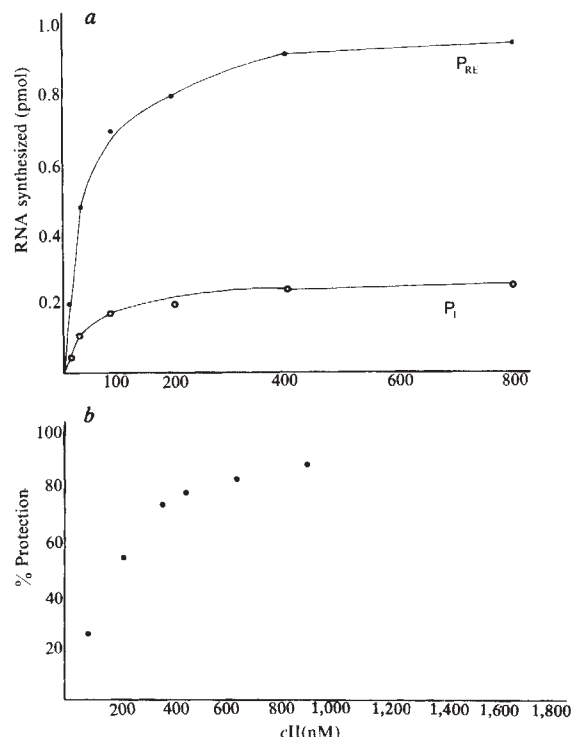


Fig. 2 *a*, Transcriptional activation response curves to cII protein for the P_{RE} and P_I promoters. *b*, DNase protection of the P_{RE} promoter in response to cII protein.

Methods: For *a*, transcription reactions were carried out *in vitro* using purified components as described previously⁵. The plasmids carrying the appropriate phage λ restriction fragments used as DNA templates are those described in ref. 5 (Fig. 3). All transcription reaction mixtures (50 μ l) contained the appropriately restricted DNA template (0.5 μ g), *E. coli* RNA polymerase holoenzyme (4 μ g; Enzo Biochemicals), the four nucleotide triphosphates (each at 200 μ M) and [α -³²P]ATP (Amersham; final specific activity 10 Ci mmol⁻¹). Purified cII protein was added at the concentrations indicated (25, 50, 100, 200, 400, 800 nM, calculated on the basis of cII being a 44,000-molecular weight tetramer⁶). Reactions were carried out at 37 °C for 20 min. The products were resolved on 5% polyacrylamide slab gels containing 8 M urea and quantitated by scintillation counting and densitometry after detection by autoradiography. For *b*, DNase I protection experiments were carried out essentially as described by Galas and Schmitz¹⁶. A 540-bp *Ava*I-*Bgl*II restriction fragment (nucleotide positions 38,215-38,754 of phage λ as determined by Sanger *et al.*²⁹) carrying the P_{RE} promoter was 5' end-labelled with ³²P at the *Ava*I site. All reactions contained 0.1 pmol of this DNA fragment dissolved in 50 μ l of 50 mM Tris-HCl, pH 8.0, 80 mM KCl, 8 mM MgCl₂, 3 mM CaCl₂, 2 mM dithiothreitol, 5% glycerol. Purified cII protein was added at the indicated concentrations (90, 220, 360, 450, 640, 900, 1,800 nM calculated as in *a*) and incubated at 23 °C for 20 min. DNase I (2 ng) was added and incubation at 23 °C was continued for 2 min. The reaction was stopped by adding 50 μ l of a solution containing 0.6 M NaOAc, 100 mM EDTA, 120 μ g ml⁻¹ carrier tRNA, followed immediately by an equal volume of phenol. The mixture was extracted twice with phenol, then twice with ether, and finally precipitated with EtOH. The precipitate was redissolved in 8 μ l of an 80% formamide gel loading buffer³⁰, heated at 95 °C for 1 min and 4 μ l applied to a standard 8% DNA sequencing gel. A typical gel pattern is shown in Fig. 3a. The amount of protection was quantitated by scanning the autoradiogram using a Zeinch laser densitometer. An identical response curve was obtained using a DNA fragment carrying the P_I promoter.

Fig. 3 Autoradiograms of DNase I protection patterns generated by cII protein binding at the P_{RE} (*a*) and P_I (*b*) promoters. The experimental details are similar to those described for Fig. 2b. In the case of P_{RE} each reaction contained 0.1 pmol of the *Ava*I-*Bgl*II fragment, 5' end-labelled at the *Ava*I site. cII protein was added as follows: lane 1, none; lane 2, 10 pmol (200 nM); lane 3, 18 pmol (360 nM); lane 4, 90 pmol (1,800 nM). In the case of P_I, a 257-bp *Hin*II-*Hha*I DNA fragment (nucleotide positions 28,929-29,185 of phage λ as determined by Sanger *et al.*²⁹) was 5' end-labelled at the *Hha*I site and 0.2 pmol was used in each reaction. The amounts of cII proteins added were as follows: lane 1, none; lane 2, 18 pmol (360 nM); lane 3, 45 pmol (900 nM); lane 4, 100 pmol (2,000 nM). Sequence assignments were made by running in parallel DNA sequence reactions carried out according to the chemical procedures of Maxam and Gilbert³⁰ using the same end-labelled fragments. The numbers indicate the nucleotide positions within the promoter relative to the transcription start-site.

example, filter binding and transcription) have failed to demonstrate any RNA polymerase interaction at the P_{RE} or P_I sites independent of cII^{5,11,12}. Perhaps the extreme sensitivity of the footprinting technique allows us to visualize a weak, but authentic interaction of RNA polymerase with these two promoters which occurs independently of cII protein. If this is the case, it suggests that RNA polymerase alone may be capable of forming weak (that is, closed) complexes at P_{RE} and P_I and that one function of cII is to shift polymerase into a stable open complex.

In contrast to the results obtained with RNA polymerase alone, addition of cII resulted in tight binding of RNA polymerase at both the P_{RE} and P_I promoters. This binding was readily detected both by standard filter assay⁵ and by footprinting procedures (Fig. 4). The region protected extends over the entire promoter signal and is typical of that expected of RNA polymerase in open complex formation. Several additional facts

are worth noting. First, RNA polymerase binding does not extend the region already protected by cII upstream of the promoter (that is, positions -44, -45; Fig. 4). Second, RNA polymerase binding causes a rather striking change in the DNase sensitivity pattern at this upstream boundary. DNA positions -44 to -46 become hypersensitive to DNase cleavage following RNA polymerase interaction (Fig. 4). This was observed both at P_{RE} and P_I and suggests that a change in DNA conformation is brought about by the cII-dependent binding of RNA polymerase. Third, it appears that the affinity of cII for the P_{RE} and P_I sites increases dramatically in the presence of RNA polymerase. That is, cII and RNA polymerase result in DNA protection at a 10-fold lower cII concentration than does cII alone (2×10^{-7} compared with 2×10^{-8} M). This increased level of affinity is also seen when measured by transcription activation (see Fig. 2a). The dramatic shift in affinity as measured either by half-maximal protection or transcription, suggests that cII

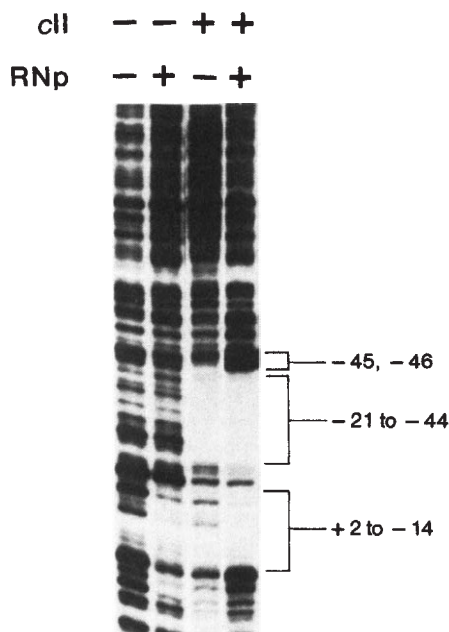


Fig. 4 DNase protection patterns generated by cII protein and/or RNA polymerase at the P_{RE} promoter. The experimental details are the same as those described for Figs 2b and 3 except for the following differences. Each reaction contained 0.07 pmol of the *AvaI*-*Bgl*II DNA fragment, 5'-end-labelled at the *AvaI* site. Where indicated, 8.0 pmol of RNA polymerase was added to the reaction. In those reactions containing RNA polymerase, cII protein was added to a final concentration of 160 nM (that is, 9 pmol), whereas when cII protein was added alone, the final concentration was 320 nM (16 pmol).

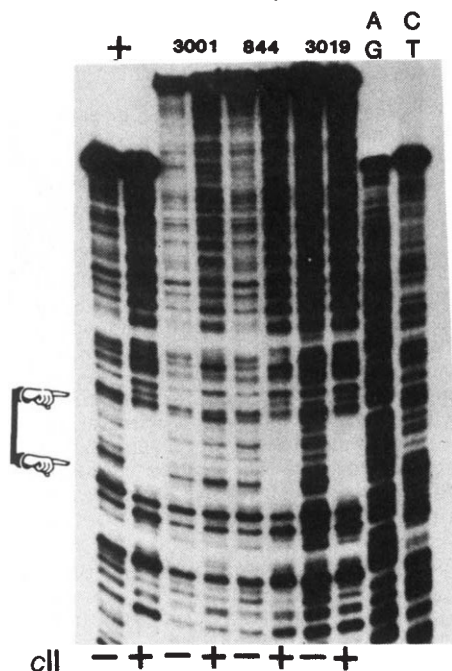


Fig. 5 DNase protection patterns generated by cII protein for various P_{RE} promoter mutations. The experimental details are the same as those described for Figs 2b and 3. For wild type (+), the 334-bp *AvaI*-*Hin*II DNA fragment was used. For each mutant, the 540-bp *AvaI*-*Bgl*II fragment was used. Fragments were 5'-end-labelled at the *AvaI* site. The representative experiments shown were carried out on the following mutants: cy3019, a point change at P_{RE} position -14; cy844, a point change at P_{RE} position -33; cy3001, a point change at P_{RE} position -40 (ref. 7). In each case, cII protein, when present, was added to a final concentration of 480 nM (25 pmol). Standard Maxam-Gilbert DNA sequencing reactions³⁰ were carried out on the wild-type fragment and used as markers. The region protected by the cII protein is indicated.

and RNA polymerase interact cooperatively at the P_{RE} and P_I signals, whereas cII binding alone exhibits no such cooperativity.

cII binds a tetranucleotide repeat

To define more precisely the DNA sequence required for cII interaction, we carried out DNase protection studies on 16 different mutations which have been characterized in the P_{RE} region⁷. Single base pair changes in both the -10 and -35 regions of P_{RE} were examined for their effects on cII binding. Typical results are shown in Fig. 5 and Fig. 7a summarizes the data.

As expected, mutations in the -10 region had no effect on cII binding. Presumably, these mutations inactivate promoter function by affecting RNA polymerase interaction at P_{RE} rather than cII interaction. In contrast, mutations in the -35 region of P_{RE} did affect cII binding. These mutations could be divided into two groups according to their cII binding abilities. Surprisingly, those mutations which eliminated cII binding fell into two distinct clusters positioned 6 bp apart (Fig. 7a). Four other mutations, which occur within the 6-bp separation between the clusters had little or no effect on cII interaction. Strikingly, this 6-bp region is positioned exactly 17 bp upstream of the P_{RE} -10 region sequence. Thus, it is exactly analogous in both size and position to the -35 region hexamer sequence found in other promoter signals²². Although this sequence at P_{RE} bears little resemblance to the consensus -35 region hexamer (TTGACA), it must carry out a similar function because single base pair changes in this region interfere with the promoter. Apparently, cII activation in some way alters the -35 region sequence specificity normally used by RNA polymerase for promoter recognition.

Most strikingly, the two clusters of P_{RE} mutations which eliminate cII binding, fall in the repeat sequence TTG-CN₆TTGC, N₆ being the hexamer sequence noted above (see Fig. 7a). Mutations affecting cII binding occur in 7 of the 8 bp which make up this tetranucleotide repeat. Note that each corresponding position of this repeat sequence is positioned 10 bp apart, exactly one turn of the DNA helix. Thus, the repeat units are aligned on one side of the DNA helix. Moreover, the six nucleotides between the repeat (N₆) comprise the -35 region recognition site for RNA polymerase. We interpret these findings to mean that the tetranucleotide repeat forms the core of the cII recognition site and that cII interaction occurs primarily on one face of the DNA. Presumably, RNA polymerase interacts with the -35 region hexamer positioned between these repeats mainly from the other side of the DNA. We point out (and discuss in detail elsewhere)⁶ that this is the first example of a transcriptional regulatory protein which interacts with a direct repeat sequence in the DNA.

cII binds the major groove

Additional evidence supporting the conclusions drawn above was obtained by examining the close apposition of cII and DNA using chemical probe experiments²³⁻²⁶. Appropriately end-labelled DNA fragments were methylated with dimethyl sulphate (DMS) either in the presence or absence of cII protein. This DNA was then cleaved at the methylated residues and analysed on polyacrylamide gels. Purine residues which were either protected from methylation or exhibited enhanced methylation in the presence of cII protein were identified (Fig. 6).

The results (summarized in Fig. 7b) indicate that all four G residues occurring within the two tetranucleotide repeat sequences were protected selectively from methylation by cII interaction. In addition, only one other G residue positioned immediately adjacent to the upstream repeat unit (that is, position -40) showed some protection. No other purine residues outside or between the repeat units were protected, including the two G residues in the -35 region hexamer sequence (at positions -31 and -35, Fig. 7b). Thus, each pair of strongly protected G residues is closely juxtaposed and the

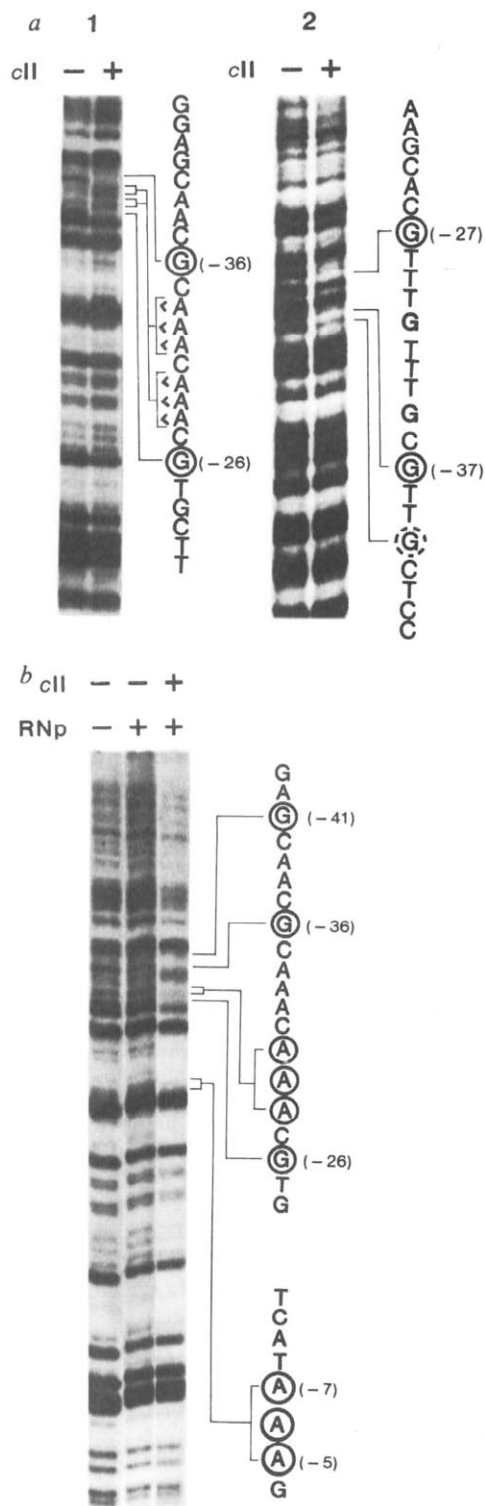


Fig. 6 (Left.) Autoradiograms of the methylation protection pattern generated by cII protein and/or RNA polymerase at the P_{RE} promoter. ○, Strongly protected residue; ◊, weakly protected residue; V, methylation enhanced residue. Numbers indicate nucleotide position in the promoter relative to the transcription start-site.

Methods: DMS protection experiments were carried out using procedures similar to those described by Siebenlist and Gilbert²⁵. To examine methylation protection on both DNA strands, two different DNA fragments were used. The P_{RE} template strand (1) was studied using the *AvaI*-*Bgl*II fragment, 5' end-labelled at the *AvaI* site, whereas the non-template strand (2) was studied using the *AvaI*-*Hin*II fragment, 5' end-labelled at the *Hin*II site. Typical reactions contained 0.1 pmol of the appropriate DNA fragment in 100 μ l of 50 mM sodium cacodylate pH 8.0, 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol. Where indicated, 100 pmol of cII and 10 pmol of RNA polymerase (RNp) were added to the reaction. The reaction was incubated at 23 °C for 20 min and then cooled to 4 °C. Then 1.0 μ l of 10.7 M DMS was added and the mixture incubated at 4 °C for 1 h. The reaction was stopped by the addition of 30 μ l of a solution containing 1.0 M 2-mercaptoethanol, 0.5 M Tris-HCl pH 7.5, 1.5 M NaOAc, 10 mM $MgCl_2$, 0.1 mM EDTA and 10 μ g of carrier tRNA. The mixture was precipitated with EtOH and the pellet dried under vacuum. This pellet was treated according to the G>A chemical sequencing procedures described by Maxam and Gilbert³⁰. The reaction products were resolved on standard 8% DNA sequencing gels

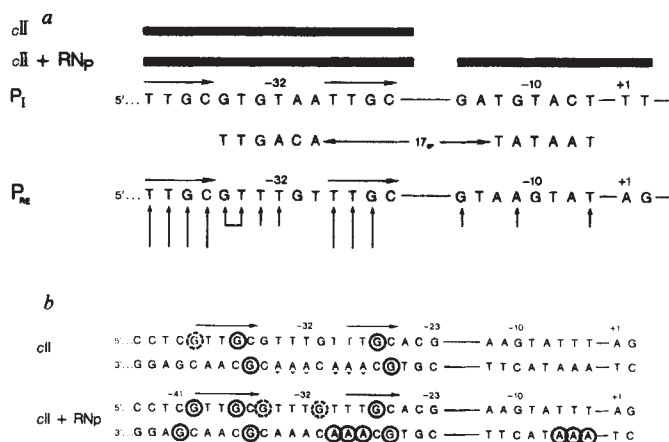


Fig. 7 (Above.) *a*, Comparison of the -10 and -35 region sequences of the P_1 and P_{RE} promoters aligned with respect to their cII-dependent transcription start-sites (+1). Only the non-template strand is shown. The consensus -10 and -35 region sequences recognized by RNA polymerase are also shown for comparison, as is the consensus 17-bp separation between them. The regions of the promoter protected from nuclease digestion by cII and cII plus RNA polymerase (RNp) are overscored by thick solid bars. The tetranucleotide repeat sequence is indicated by horizontal arrows. The long-stemmed vertical arrows indicate those residues which when mutated affect cII binding. Short-stemmed arrows indicate those positions which when mutated have no effect on cII binding. This figure summarizes the footprinting data (as in Figs 3-5) generated with the following cy and cII mutations: 3003, 42, 3008, 3072, 3077, 3098, 3107, 3095, 3078, 3075, 3019, 3048, 3071, 3085, 3109 and 3114 (refs 4, 7, 8, 10). *b*, Summary of the methylation protection studies carried out with cII alone and cII plus RNA polymerase (RNp) at the P_{RE} promoter (as shown in Fig. 6). Sequences aligned as in *a* and all designations are the same as those used in Figs 6 and 7a.

two repeat units are aligned perfectly on one face of the DNA. As DMS methylation is known to be selective for the N-7 position of the guanine base located in the DNA major groove²⁶⁻²⁸, cII interaction must occur primarily within this major groove region.

In contrast to the G methylation pattern, the A residues positioned between the protected G residues showed dramatically enhanced methylation in the presence of cII protein (Fig. 6a and summarized in Fig. 7b). No other purines within or outside this region were affected. Methylation of A residues occurs at the N-3 position in the minor groove of duplex DNA²⁶⁻²⁸. Thus, enhanced methylation at these positions suggests that cII binding, rather than protecting the minor groove,

makes this groove more accessible to DMS attack. One possibility is that cII interaction results in a DNA conformation change which opens the minor groove, thereby increasing the reactivity of the adenine N-3 position. Another possibility is that cII binding leads to local denaturation of the DNA and that the increased methylation results from adenine N-1 methylation, a highly reactive position not normally available for reaction on duplex DNA. A third possibility, although less likely, is that the enhanced methylation is simply the result of a localized increase in DMS concentration brought about by protein binding. In any case, the methylation protection results are completely consistent with both the DNase protection experiments and the mutational analyses. The cII tetramer clearly recognizes a repeating DNA sequence making its contacts primarily in the major groove on one face of the DNA helix.

Chemical protection experiments also were carried out in the presence of cII and RNA polymerase. The four G residues strongly protected by cII alone were also protected in the presence of RNA polymerase (Figs 6b, 7b). This suggests that cII remains bound to the DNA even after RNA polymerase binds. Alternatively, but less likely, the RNA polymerase replaces cII, making identical contacts with these four G residues. More importantly, RNA polymerase binding results in strong protection of other purine residues positioned both outside and between the tetranucleotide repeat sequence as well as in the -10 region. For example, RNA polymerase protects the G residues at positions -31 and -41. These positions are located on the side of the DNA opposite to that which cII binds. Furthermore, no enhanced methylation is observed and, in particular, the three contiguous A residues at positions -28 to -30 are now strongly protected (Figs 6b, 7b). These minor groove positions again are located on the far side of the helix. Apparently, RNA polymerase forms contacts with the region between and around the two repeat sequences and does so primarily from the other side of the DNA. A similar picture is obtained if we superimpose the contact sites made by RNA polymerase at other promoters onto the P_{RE} sequence (aligned relative to the -10 region hexamer)²⁶. Again, the RNA polymerase contacts occur opposite to those found to cII. Thus, all the evidence, direct and indirect, indicates that cII and RNA polymerase both interact with DNA in the -35 region of the P_{RE} and P_I promoters by making contact with different sides of the DNA helix, thereby sandwiching the DNA between them. This close juxtapositioning of cII and RNA polymerase in the -35 recognition region of the promoter strongly suggests

that transcriptional activation will involve direct interaction between these two protein moieties.

Conclusion

We have demonstrated that the transcriptional activator cII is a DNA binding protein which recognizes homologous sequences in the -35 regions of the P_{RE} and P_I promoters. Recognition centres in a tetranucleotide repeat sequence, each repeat unit being aligned on the same side of the DNA one turn of the helix apart. cII-DNA contacts occur primarily in the major groove and similar contacts are made in each repeat segment. The tetranucleotide repeats are separated by 6 bp which comprise the -35 region recognition sequence for RNA polymerase. Polymerase makes sequence-specific contacts with this hexamer and these occur primarily on the side of the helix opposite to that used by cII for interaction. Most strikingly, the -35 region hexamer of the cII-dependent promoter bears little resemblance to the sequence usually recognized by RNA polymerase. Apparently, cII interaction allows RNA polymerase to use this markedly different -35 region. Moreover, these promoters also contain rather poorly conserved -10 region hexamer sequences. Again, cII activation results in an efficient utilization of this sequence by the polymerase. Presumably, cII accomplishes these functions by changing the local DNA conformation and/or by direct protein-protein interactions with the polymerase.

We emphasize that this positive regulatory system lends itself especially well to a more detailed analysis of transcriptional activation. Our ability to overproduce and purify large quantities of the cII activator has allowed both biochemical and physical characterization of this protein and crystallographic analysis has now been initiated. In addition, more than 100 independent mutations have been introduced into the cII gene (97 codons) and many of these variants are now being characterized structurally. We have already cloned, overproduced and are in the process of isolating and analysing more than a dozen of these single amino acid variants of cII. Each cII derivative is being characterized as to its ability to form tetramers, bind DNA, and/or activate transcription. Clearly, analyses such as these, both at the level of the DNA signal and on the activator protein itself, should help to elucidate further the molecular mechanisms involved in transcription activation.

We thank Michael Mahoney for helping to prepare the mutant phage DNAs, and Linda Hampton for typing and editing the manuscript.

Received 17 March; accepted 21 June 1983.

- Reichardt, L. & Kaiser, A. D. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2185-2189 (1971).
- Echols, H. & Green, L. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2190-2194 (1971).
- Herskowitz, I. & Hage, D. A. *Rev. Genet.* **14**, 399-445 (1980).
- Wulff, D. L. & Rosenberg, M. in *The Bacteriophage Lambda* Vol. 2, 1983 (eds Hendrix, J., Roberts, J., Stahl, F. & Weisberg, R.) (Cold Spring Harbor Laboratory, New York, in the press).
- Shimatake, H. & Rosenberg, M. *Nature* **292**, 128-132 (1981).
- Ho, Y. S., Lewis, M. & Rosenberg, M. *J. biol. Chem.* **257**, 9128-9134 (1982).
- Wulff, D. L., Mahoney, M., Shatzman, A. & Rosenberg, M. *Proc. natn. Acad. Sci. U.S.A.* (submitted).
- Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. *Nature* **272**, 414-423 (1978).
- Wulff, D. L. *et al. J. molec. Biol.* **138**, 209-230 (1980).
- Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. in *The Operon* (eds Miller, J. H. & Reznikoff, W. S.) 304-324 (Cold Spring Harbor Laboratory, New York, 1978).
- Schmeissner, U., Court, D., Shimatake, H. & Rosenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3191-3195 (1980).
- Schmeissner, U. *et al. Nature* **292**, 173-175 (1981).
- Abraham, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 2477-2481 (1980).
- Hoess, R. H., Foeller, C., Bidwell, K. & Landy, A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2481-2486 (1980).
- Davies, R. W. *Nucleic Acids Res.* **8**, 1765-1782 (1980).
- Galas, D. & Schmitz, A. *Nucleic Acids Res.* **5**, 3157-3170 (1978).
- Schmitz, A. *Nucleic Acids Res.* **9**, 277-292 (1981).
- Taniguchi, T., O'Neill, M. & deCrumbrugghe, B. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5090-5094 (1979).
- Queen, C. & Rosenberg, M. *Nucleic Acids Res.* **9**, 3365-3377 (1981).
- Sakonju, S. & Brown, D. *Cell* **31**, 395-405 (1982).
- Brent, R. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4204-4208 (1981).
- Rosenberg, M. & Court, D. *A. Rev. Genet.* **13**, 319-353 (1979).
- Gilbert, W., Mayam, A. & Mirzabekov, A. *Alfred Benzon Symp.* **13**, 139-148 (1976).
- Johnson, A., Meyer, B. J. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1783-1787 (1978).
- Siebenlist, U. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 122-126 (1980).
- Siebenlist, U., Simpson, R. & Gilbert, W. *Cell* **20**, 269-281 (1980).
- Singer, B. *Prog. Nucleic Acids Res. molec. Biol.* **15**, 219-284 (1975).
- Lawley, P. D. & Brooks, P. *Biochem. J.* **89**, 127-135 (1963).
- Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F. & Peterson, G. B. *J. molec. Biol.* **162**, 729-773 (1982).
- Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).

Discovery of an IR echo from a supernova dust cloud

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Supernova (SN) 1982g in NGC1332 was discovered by Maza on 28 March 1982 at $m_{\text{ps}} = 14.0$ (ref. 1) some 30 kpc ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) from the nucleus of the parent galaxy (distance, 30 Mpc; type, SO)². No other optical data have been reported so the SN type is uncertain, although no Type II SN has ever been identified in a galaxy earlier than SA³. We have obtained IR light curves for SN 1982g covering 100–250 days after its discovery. We show here that the IR radiation is due to heated dust. No evidence has been found for grain condensation in the SN ejecta. In fact, the light curves reveal that the IR emission originates from a pre-existing circumstellar dust cloud and is produced by an 'echo' of the UV and visible pulse of the SN.

IR observations of the SN were carried out at the 3.9-m Anglo-Australian Telescope and the 3.8-m United Kingdom Infrared Telescope (Hawaii) in the J (1.25 μm), H (1.65 μm) and K (2.2 μm) bands (plus a single upper limit at L (3.4 μm)) (see Fig. 1). If we use the typical optical colours of a SN to specify the temperature of a black body spectrum which we then extrapolate into the IR, a much lower IR flux is deduced than that which we observed. For example, at 100 days the SN is at least 3 mag brighter at K than such an extrapolation would predict. The IR colours $J-H$ and $H-K$ indicate that this excess is due to a continuum with a colour temperature of 1,170 K at 100 days which cools gradually to 920 K at 200 days. SN 1979c and 1980k, both Type IIs are also known to have developed IR excesses^{4,5}. For SN 1982g galactic interstellar reddening is negligible at JHK because it is of high galactic latitude ($b = -50^\circ$). The large distance from the nucleus of the parent galaxy ensures that the reddening, as well as any stellar contribution to the IR flux, due to NGC1332, is also unimportant. In any case, dust in any location could not render the SN to produce the observed IR colours without rendering it optically undetectable.

We have considered three possible mechanisms for the IR emission. These are free-free emission from SN shock-heated circumstellar gas⁶, thermal emission from grains formed in the SN ejecta^{7,8}, and thermal emission from pre-existing circumstellar grains⁹. Only the last of these provides a convincing interpretation of the data. It is possible that the SN progenitor was surrounded by an optically-thin gas shell formed by the progenitor wind. This gas would be shock-heated by the SN, producing a free-free contribution to the IR flux. However, it is implausible that a significant fraction of the observed IR radiation arises from this mechanism as it would be accompanied by a radio flux ~ 50 stronger than that which seems to be typical for SN¹⁰. If the gas were optically thick ($\tau_{\text{IR}} \geq 1$) then unlikely mass loss rates of $\dot{M} > 10^{-2} M_\odot \text{ yr}^{-1}$ from the progenitor would be needed to produce the necessary gas densities. Emission from dust formed in the ejecta can also be dismissed. A thermal source must be at least as large as a black

body of the same luminosity and temperature. At 100 days the appropriate black body radius is $1.9 \times 10^{16} \text{ cm}$, implying an ejection velocity of $23,000(H_0/50)^{-1} \text{ km s}^{-1}$ for the emitting material. However, Doppler widths of lines indicate that the outermost layers of SN reach velocities of up to only $10,000 \text{ km s}^{-1}$ (ref. 11). Furthermore, conditions favourable for rapid grain growth only exist much deeper in the SN in the slower moving mantle⁸.

The large SN to grain distances required to account for the observed IR flux suggests that dust formation must pre-date the SN explosion. If the progenitor were surrounded by an extensive dust cloud similar to that around Betelgeuse¹² (radius, $R = 0.1 \text{ pc}$; $\tau_{\text{UV-VIS}} = 0.15$) a substantial amount of the UV-visible pulse of the SN will be converted into IR radiation. Given the extended nature of the dust cloud, light travel times across it must be considered and so one might expect the geometry of the emission to be similar to that of the UV-fluorescence models of Morrison and Sartori¹³. This provides a natural explanation for the IR light curves. Bode and Evans⁹ have adapted this 'echo' model to describe the general case of a variable light source within a dust cloud. The SN flash of UV and visible radiation propagates outwards into the cloud. Since the bolometric light curve is sharply peaked^{14,15} we can approximate the time dependence of the luminosity to a top hat function,

$$L_{\text{SN}} = \begin{cases} 10^{43} \text{ erg s}^{-1} & 0 < t < 10 \text{ days} \\ 0 \text{ erg s}^{-1} & t > 10 \text{ days} \end{cases}$$

where t is the time after the outburst, which is assumed to have occurred within a few days of discovery. As a consequence of this narrow pulse only the thin shell of dust (10 light days thick) coincident with this pulse of energy is hot at any given instant.

The temperature of the dust can be used to determine the SN to hot grain distance by means of the radiation balance for

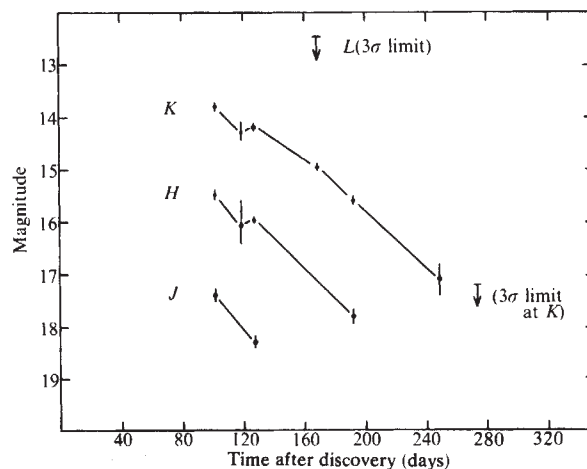


Fig. 1 IR light curves of SN 1982g.

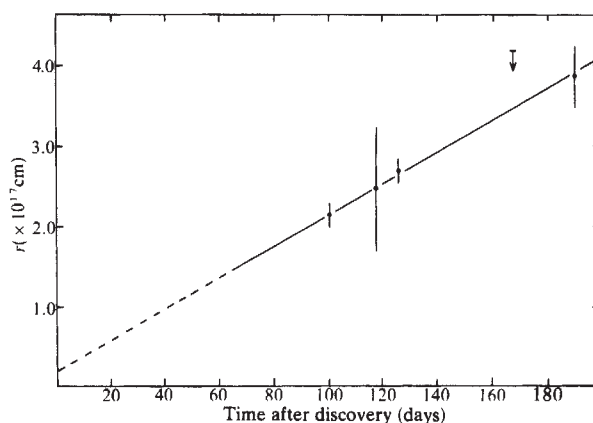


Fig. 2 Distance, r , of heated grains from SN as a function of time.

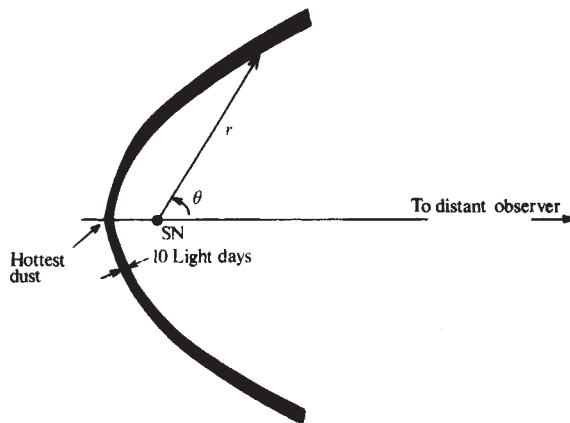


Fig. 3 The paraboloidal shell $r = ct/(1 - \cos \theta)$ of hot dust from which a distant observer receives IR radiation.

the grains,

$$\frac{L_{\text{SN}}}{4\pi r^2} \bar{Q}(a, T_{\text{SN}}) \pi a^2 = 4\pi a^2 \bar{Q}(a, T_g) \sigma T_g^4 \quad (1)$$

where r is the SN-hot grain distance, $\bar{Q}(a, T)$ the Planck average emissivity, a the grain radius, T_{SN} the colour temperature of the SN photosphere, T_g the grain temperature, derived from the IR colours assuming a λ^{-1} emissivity law, and σ Stefan's constant. We have assumed that the optical properties of the grains are described by $\bar{Q}/a \propto T_g$. For illustration we have used the values for $\bar{Q}$ quoted by Draine¹⁶ for carbon. (Use of a dielectric grain material such as silicate¹⁶ does not affect our conclusions.) Rearranging equation (1) and substituting typical numerical values, we obtain

$$r = 7.2 \times 10^{24} T_g^{-5/2} \left(\frac{a}{0.1 \mu\text{m}} \right)^{-1/2} \left(\frac{L_{\text{SN}}}{10^{43} \text{ erg s}^{-1}} \right)^{1/2} \text{ cm} \quad (2)$$

The observed change in grain temperature is interpreted as being due to the increasing distance, r , at which the heated grains lie. The distances inferred by this method are presented in Fig. 2, plotted against time. A straight line fits these points well, and gives as the gradient a constant velocity

$$v = (2.2 \pm 0.2) \times 10^5 \left(\frac{a}{0.1 \mu\text{m}} \right)^{-1/2} \left(\frac{L_{\text{SN}}}{10^{43} \text{ erg s}^{-1}} \right)^{-1/2} \text{ km s}^{-1} \quad (3)$$

For typical values of a (ref. 17) and L_{SN} (ref. 14) this velocity is comparable to the speed of light. The echo model predicts this result. In general when a source 'switches on' in a dust cloud, light travel-time arguments show⁹ that the grains from which a distant observer receives IR emission lie within a paraboloid of revolution $r = ct/(1 - \cos \theta)^{-1}$ (Fig. 3). For a pulse-like light curve this heated dust is confined within the shell as shown in Fig. 3. For a given paraboloid the temperature distribution is such that the hottest dust is situated at the vertex ($r = ct/2$, $\theta = \pi$), the point closest to the SN. Integrating the flux from the paraboloidal shell, one can show that nearly all the flux comes from this hot region around the vertex providing that the observations are made on the Wien slope of the thermal emission; for example, if the dust at the vertex has a temperature of 1,000 K and observations are made at $2.2 \mu\text{m}$, 90% of the flux comes from within 30° of the vertex. Cooler grains further from the SN do not contribute significantly to our measurements. The expected recession velocity of the vertex

$$\frac{\partial}{\partial t} \left(\frac{ct}{1 - \cos \theta} \right) \Big|_{\pi} = \frac{c}{2}$$

consistent with the determined velocity v . In addition, extrapolation of the graph back to $t = 0$ gives $r = 0$ within the errors. The linearity, gradient and (0, 0) intercept of the r versus t graph are all strong evidence for the echo model. Detailed numerical modelling (J.R.G. *et al.*, in preparation) of a SN embedded in a Betelgeuse-type dust cloud of $\tau_{\text{UV-VIS}} \sim 0.1$,

mass $\sim 10^{-2} M_\odot$ and radius $\sim 10^{18}$ cm shows that the observed IR light curves are reproduced.

We conclude that the IR emission from SN 1982g was due to a pre-existing circumstellar dust shell heated by the SN pulse. This is the first clear evidence for the occurrence of an IR echo in any source.

We thank Dr Bob Joseph and Dr Norna Robertson for valuable comments and suggestions, and Mr David King for efficient measuring of star positions. We are also grateful for the support provided by the staffs of the AAO, RGO and UKIRT. J.R.G. thanks the Department of Education for Northern Ireland for a research studentship and travel funds. G.P. thanks the University of Keele for a research studentship and the SERC for travel funds. M.F.B. is supported by the US Department of Energy.

Note added in proof; Dwek¹⁸ has recently interpreted the IR light curves of both SN 1979c and SN 1980k as being due to an echo of the UV-visual output of the SN.

Received 25 May; accepted 6 July 1983.

1. Maza, J. *IAU Circ. No.* 3684 (1982).
2. de Vaucouleurs, G. & de Vaucouleurs, A. *Second Reference Catalogue of Bright Galaxies* (University of Texas Press, 1976).
3. Cowal, C. T. *The Palomar Supernova Search Master List* (Caltech, 1982).
4. Merrill, K. *IAU Circ. No.* 3444 (1980).
5. Dwek, E. *et al. Bull. Am. astr. Soc.* **13**, 795 (1981).
6. Chevalier, R. A. *Astrophys. J. Lett.* **251**, 259-265 (1981).
7. Cernuschi, E., Maricano, F. & Codina, S. *Ann. Astrophys.* **30**, 1039-1051 (1967).
8. Hoyle, F. & Wickramasinghe, N. C. *Nature* **226**, 62-63 (1970).
9. Bode, M. F. & Evans, A. *Astr. Astrophys.* **73**, 113-120 (1979).
10. Weiler, K. W., Sramek, R. A., van der Hulst, J. M. & Panagia, N. in *Supernovae: A Survey of Current Research*, 281-291 (Reidel, Dordrecht, 1982).
11. Branch, D. *et al. Astrophys. J.* **244**, 780-804 (1981).
12. McMillan, R. S. & Tapia, S. *Astrophys. J. Lett.* **226**, L87-L89 (1978).
13. Morrison, P. & Sartori, L. *Astrophys. J.* **158**, 541-570 (1969).
14. Colgate, S. A., Petschek, A. K. & Kriese, J. T. *Astrophys. J. Lett.* **237**, L81-L85 (1980).
15. Panagia, N. *et al. Mon. Not. R. astr. Soc.* **192**, 861-879 (1980).
16. Draine, B. T. *Astrophys. J.* **245**, 880-890 (1981).
17. Rowan-Robinson, M. & Harris, S. *Mon. Not. R. astr. Soc.* **200**, 197-215 (1982).
18. Dwek, E. *Astrophys. J.* (in the press).

A two-stage mechanism for escape of Na and K from Io

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It is generally accepted that Io is the source of S, O, Na and K which, after ionization, form the constituents of the Io plasma torus. The escape of S and O from Io can be understood in terms of the photochemistry of a predominantly SO_2 atmosphere created by the high vapour pressure of SO_2 (refs 1, 15). However, the vapour pressures of Na_2S , K_2S and other common compounds containing Na and K are negligible at the surface temperatures of Io. This has given rise to the suggestion that over part of Io's surface (the nightside) the atmosphere is thin enough so that surface sputtering by co-rotating ions can eject Na and K directly into the Io torus^{2,3}. The main objection to this idea is that it implies a 'Sun-locked' source for Na and K, while observations of the Na and K clouds around Io indicate a 'Jupiter-locked' ejection mechanism. We propose here that Na and K escape from Io in two stages. Atoms of Na and K are first sputtered into the atmosphere from the surface by high-energy magnetospheric ions. Atmospheric sputtering⁴ by low-energy co-rotating ions then removes these constituents (along with others present) out of Io's gravitational field. We suggest that the observed Na and K ejection asymmetry is due to preferential sputtering of atmospheric particles on the hemisphere of Io facing Jupiter. The estimated injection rates are sufficiently large to maintain the observed K, Na, and O clouds observed around Io^{5-7,18}.

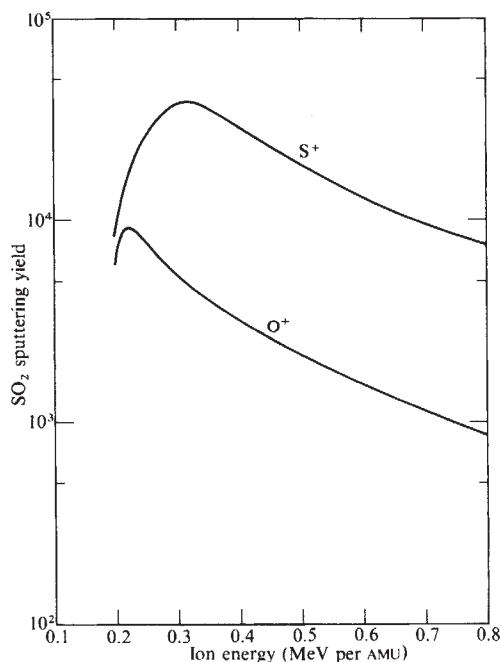


Fig. 1 The calculated SO_2 sputtering yield as a function of incident energy for S^+ and O^+ ions. The calculations were performed using the theory of Watson and Tombrello¹².

The SO_2 abundance of 0.2 cm atm detected by the Voyager I IRIS experiment in the subsolar region of Io corresponds to a SO_2 vertical column density $N_{\text{IRIS}} \approx 5 \times 10^{18} \text{ cm}^{-2}$ (ref. 8). This is approximately the atmospheric SO_2 abundance that would exist if the SO_2 gas were in thermodynamic equilibrium with SO_2 surface frost at $T = 130 \text{ K}$. The globally-averaged SO_2 vertical column density $\bar{N}$ is probably much less than this value since the vapour pressure of SO_2 decreases exponentially with decreasing temperature. An estimate of $\bar{N}$ may be obtained by assuming local pressure equilibrium between atmospheric SO_2 and surface frost. From an extrapolation of the SO_2 vapour pressure data of D. D. Wagman (personal communication), and a surface temperature variation which approximates the IRIS observations⁸, we obtain $\bar{N} \approx 5 \times 10^{17} \text{ cm}^{-2}$. Note that photochemical models of Io's atmosphere which reproduce the characteristics of the downstream ionosphere as observed by Pioneer 10 require $\bar{N} \geq 1 \times 10^{17} \text{ cm}^{-2}$ (ref. 9).

Co-rotating S^+ and O^+ ions in the plasma torus impacting Io's atmosphere are unable to penetrate to Io's surface if $\bar{N} \geq 10^{16} \text{ cm}^{-2}$ (ref. 10). In order for S^+ and O^+ ions to penetrate an SO_2 atmosphere characterized by $\bar{N} \approx 5 \times 10^{17} \text{ cm}^{-2}$, their incident kinetic energies must be greater than 0.3 MeV and 0.1 MeV, respectively. The Voyager I low-energy charged particle (LECP) and plasma science (PLS) experiments detected a flux $F \approx 10^5 \text{ cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1}$ of ions in the vicinity of Io's orbit with energies greater than these minimum values¹⁰.

The sputtering of frozen SO_2 frost by high-energy ($0.08 \leq E$ (MeV per AMU) ≤ 1.30) He and F ions has recently been studied by Melcher *et al.*¹¹ (see also ref. 10). The peak sputtering yield for incident F^+ was 7,300 at energy $E_p = 0.32 \text{ MeV per AMU}$. The yield spectrum exhibited a rapid rise with increasing energy for $E < E_p$, and an exponential decrease for $E > E_p$. The qualitative character of the yield spectrum was previously predicted on the basis of a modified lattice potential model of high-energy ion erosion developed by Watson and Tombrello¹². The theory correctly predicts the energy at which the peak yield occurs; however, the magnitude of the predicted yields is a factor of ~ 3 too large. We have utilized this theory to estimate the sputtering yield due to impacting high energy O^+ and S^+ on SO_2 . In Fig. 1 we show the calculated yields as a function of incident ion energy, E , made using the same normalization

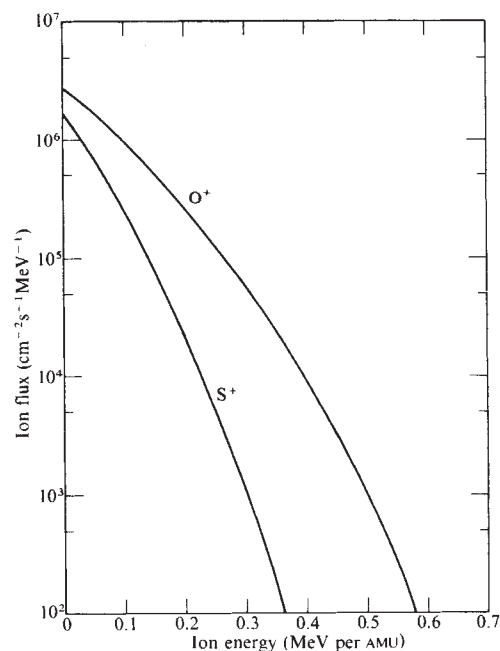


Fig. 2 The differential ion flux in the vicinity of Io's orbit, adopted from Lanzerotti *et al.*¹⁰, and extrapolated to higher energies using the power law $d(\text{flux})/dE = (E/E_0)^{-n}$, where E_0 is a reference energy and $n = 7$.

factor that adjusted the calculated F^+ sputtering yields to agree with the data. The peak yield for O^+ on SO_2 frost is 8,900 and occurs at $E = 0.23 \text{ MeV per AMU}$. The sputtering yield spectra for F^+ and O^+ are similar because they have similar masses. The peak yield for S^+ on SO_2 frost is 35,000 at $E = 0.4 \text{ MeV per AMU}$.

The surface sputtering rate due to impacting ions is given by the integral of the product of the yield and the differential ion flux incident on the surface. The differential ion flux in the vicinity of Io's orbit was measured by the Voyager I PLS and LECP experiments; however, the composition of the high-energy plasma is uncertain. We have used the PLS and LECP data and data extrapolations of the high-energy ion flux given by Lanzerotti *et al.* (ref. 10, and see Fig. 2), to calculate the surface sputtering rate on Io. Assuming that the high-energy ions consist of S^+ and O^+ in the ratio 1:2, we find a surface sputtering rate $\phi_{\text{SO}_2} \approx 8 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$. This value of the flux of SO_2 molecules into the atmosphere is about 7 orders of magnitude smaller than the sublimation rate of SO_2 in the subsolar region of Io. Thus, surface sputtering by these high-energy ions is insignificant for the overall mass balance of the atmosphere. If minor constituents such as Na and K are present on the surface with the SO_2 frost, then sputtering may be an important mechanism for mobilizing these constituents.

It has been suggested that Na and K exist on the surface of Io in the form of Na_2S and K_2S (ref. 13); however, the surface abundances of these salts are unknown. We consider the consequences of having K, Na, and S present on the surface in cosmic proportions. This assumption implies a number ratio of Na atoms to S atoms of 0.12, and a ratio of K to S of 0.008 (ref. 14). In the sputtering process we assume that each surface constituent is ejected in proportion to its surface abundance. Thus, we estimate the surface source of Na and K to be $\phi_{\text{Na}} \approx 1 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ and $\phi_{\text{K}} \approx 6 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$. These fluxes are of the correct magnitude to maintain in a steady state the Na and K clouds observed around Io⁵⁻⁷.

Once in the atmosphere Na and K will escape from Io by the same mechanism that operates for S and O. Earth-based spectroscopic observations of the neutral Na and K clouds indicate particle injection velocities from Io $\geq 3 \text{ km s}^{-1}$ (refs 6, 7, 19). Thermal Jean's escape will produce escaping K with

velocities greater than this minimum value only if the exosphere temperature $T_{\text{ex}} \geq 14,000$ K, while models of Io's ionosphere indicate $T_{\text{ex}} \approx 1,000$ K (ref. 1). Sputtering of atmospheric particles by impacting co-rotating S^+ and O^+ will produce an average velocity for the escaping particles $\geq 2.6 \text{ km s}^{-1}$. There are simple and compelling reasons to believe that the exosphere of Io is dominated by neutral oxygen atoms¹ and that Io's exobase lies at more than 2.0 Io radii from the surface¹⁵. The yield for atmospheric sputtering by low-energy ions is $Y \approx 1$ (ref. 4). The flux of co-rotating ions averaged over the Io plasma torus is $F_{\text{CR}} \approx 10^{10} \text{ cm}^{-2} \text{ s}^{-1}$ (ref. 10). The escaping flux due to atmospheric sputtering is therefore $F_{\text{escape}} \geq 9 \times 10^{27} \text{ O atoms s}^{-1} \approx 200 \text{ kg s}^{-1}$. This is sufficient to supply the neutral oxygen cloud that exists along the orbit of Io¹⁶. Sulphur atoms will be sputtered from the atmosphere in approximately the same proportion in which they exist in the atmosphere at the exobase.

Voyager I measurements of the plasma torus indicate a large but negative radial gradient in the concentration of corotating ions across the orbit of Io¹⁷. This is of key importance since the flux of sputtered atoms is proportional to the flux of incoming ions. The observed gradient in ion density (and hence co-rotating flux) is sufficiently large that if Io's exobase is located

at 2.0 Io radii from its surface, then the flux of sputtered atoms due to impacting S^+ and O^+ is a factor of ~ 2 greater on Io's inner hemisphere (facing Jupiter) than on the outer hemisphere. Therein may lie a particularly simple explanation of the Na and K ejection asymmetry¹⁸⁻²⁰. If Na and K can be supplied to the atmosphere at the required rate, then there should be greatly enhanced atmospheric sputtering of Na, K, O, and S on Io's inner hemisphere. This would lead to ejection asymmetries not only for Na and K (observed) but also for S and O (as yet unobserved).

Many questions remain to be answered in order to understand the details of this two-stage mechanism. For example, how are Na and K species affected by photochemistry and diffusion? What fraction of the atoms sputtered from the surface eventually escape into the torus? What are the characteristics of the asymmetric escape of S and O? These questions provide the basis of a study that is in progress.

We thank J. Trauger, A. Dessler, and A. Summers for helpful discussions; and K. Cherrey for calculations. This research was supported by NASA grants NAGW-202 and NAGW-313. Contribution no. 3873 from the Division of Geological and Planetary Sciences, California Institute of Technology.

Received 11 May; accepted 28 June 1983.

1. Kumar, S. *J. geophys. Res.* **87**, 1677-1684 (1982).
2. Haff, P. K. *et al. J. geophys. Res.* **86**, 6933-6938 (1981).
3. Kumar, S. & Hunten, D. M. in *Satellites of Jupiter* (ed. Morrison, D.) 782-806 (University of Arizona Press, 1982).
4. Haff, P. & Watson, C. C. *J. geophys. Res.* **84**, 8436-8442 (1979).
5. Macy, W. & Trafton, L. *Astrophys. J.* **200**, 510-519 (1975).
6. Brown, R. A. & Yung, Y. L. in *Jupiter* (ed. Gehrels, T.) 1102-1145 (University of Arizona Press, 1976).
7. Trafton, L. *Astrophys. J.* **247**, 1125-1140 (1981).
8. Pearl, J. *et al. Nature* **280**, 755-758 (1979).
9. Kumar, S. *Geophys. Res. Lett.* **7**, 9-12 (1980).
10. Lanzerotti, L. J. *et al. Astrophys. J.* **259**, 920-929 (1982).
11. Melcher, C. L. *et al. Geophys. Res. Lett.* **9**, 1151-1154 (1982).
12. Watson, C. C. & Tombrello, T. A. *Radiat. Effects* (submitted).
13. Fanale, F. P. *et al. in Satellites of Jupiter* (ed. Morrison, D.) 756-781 (University of Arizona Press, 1982).
14. Lang, K. R. *Astrophysical Formulae* (Springer, Berlin, 1978).
15. Summers, M. E. & Yung, Y. Preprint (California Institute of Technology, 1983).
16. Shemansky, D. & Smyth, W. J. *Geophys. Res.* (submitted).
17. Belcher, J. W. in *Physics of the Jovian Magnetosphere* (ed. Dessler, A.) 68-105 (Cambridge University Press, 1983).
18. Smyth, W. H. & McElroy, M. B. *Astrophys. J.* **226**, 336-346 (1978).
19. Carlson, R. W. *et al. Geophys. Res. Lett.* **10**, 469-472 (1975).
20. Matson, D. L. *Science* **199**, 531-533 (1978).

A new radar auroral backscatter experiment

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In 1982 a new radar auroral backscatter system, SABRE (Sweden and Britain Radar Experiment), commenced operation in northern Europe. Observations with this ground-based system allow estimates to be made of the electric fields in the auroral E-region. As an electric field probe, the coherent radar technique is unique in that measurements are made over a large area ($\sim 200,000 \text{ km}^2$) with good spatial resolution ($20 \times 20 \text{ km}^2$) and with a temporal resolution of typically 20 s. The system field of view covers an L-value range 4.3-5.8. This region in the magnetosphere-ionosphere system is now accessible for the first time for detailed electric field studies. We present here observations of two pulsation events.

The auroral radars are sensitive to electrostatic plasma waves in the E-region ionosphere. The mean Doppler shift of the frequency of the transmitted radar signal is closely related to the phase velocity of these waves. The simple fluid theory¹ predicts that the phase velocity in a given direction is equal to the component of the electron drift velocity in that direction. Thus, an analysis of Doppler shifts, observed with radars in a given volume from two independent directions, allows an estimate to be made of the electron drift velocity. Because electrons are $\mathbf{E} \times \mathbf{B}$ drifting at E-region heights, the electric field vector can also be estimated².

Recent, more detailed solutions of the basic equations, which govern the plasma waves excited by the two-stream instability³⁻⁵, predict a different, but still deterministic, relationship between the electric field and the observed Doppler shift. Furthermore, new experimental evidence supports qualitatively the predictions of the new theories⁶. As the relationship between the Doppler shifts and the electric field becomes better determined, improved approximations of the latter can be made.

The radar auroral technique used in the SABRE system is similar to the one already used in the STARE (Scandinavian Twin Auroral Radar Experiment) system⁷. This type of system consists of two bi-static radar stations. The Max-Planck Institut für Aeronomie (MPAE), FRG, and Leicester University (LU), UK, have each built one of these radar stations⁸. The German radar is located near Uppsala, Sweden and is operated by MPAE in cooperation with the Uppsala Ionospheric Observatory. The British radar is located near Wick, Scotland, and is operated by LU.

The German radar operates on 142.585 MHz and the British radar on 153.2 MHz. Thus, the radars are sensitive to plasma waves with 1.05 and 0.98 m wavelength, respectively. The lobes from the two transmitter antenna and from the two receiver antenna cover a large common area of the auroral ionosphere ($\sim 200,000 \text{ km}^2$). The radar operations are characterized by the parameters listed in Table 1. The accuracy of the directional measurements depends critically on the effectiveness of the isolation between the receiver antenna lobes. The horizontal radiation pattern for the German receiver antenna is reproduced in Fig. 1 and very distinct narrow lobes are evident. In normal operation a sequence consisting of a single-pulse followed by a double-pulse is transmitted with a repetition frequency of 50 MHz. This sequence is continuously executed during the integration time. The averages of the measurements are finally formed and combined⁷⁻⁹ to yield the backscattered signal intensity (from single pulse pattern) and the mean radial Doppler shift (from double pulse pattern) as a function of lobe

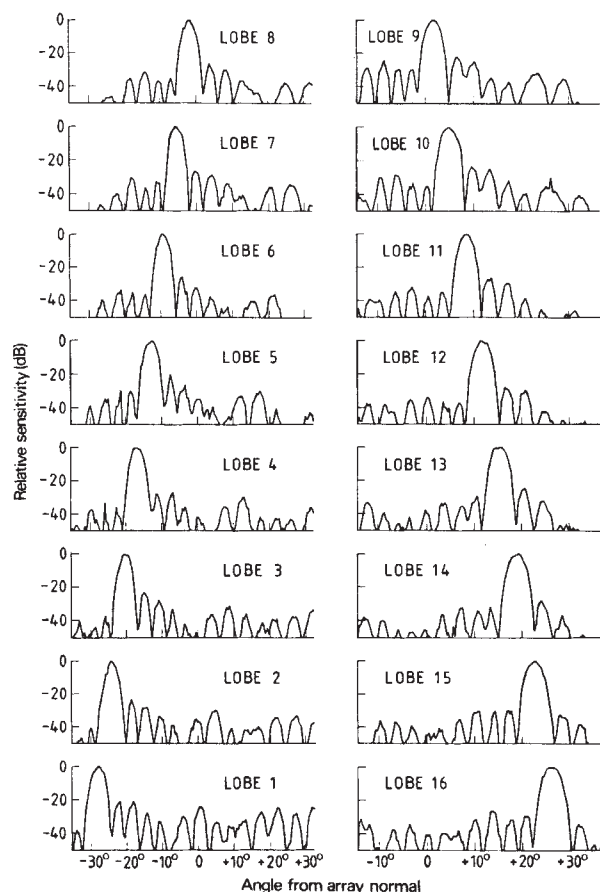


Fig. 1 The horizontal radiation pattern of the 16 individual lobes of the German receiving array was experimentally determined by placing a small transmitter on a plane flying along a circular path centred at the radar station.

and range. The British radar has additional features, which allow real time control of the radar and transfer of data in real time. The data tapes from the two stations are analysed at both MPAE and LU; the Doppler shifts are combined to yield estimates of the electron drift velocity in a series of geographical locations (grid points). At a given grid point we combine the measurements from the two radars which were made in the lobe range cell containing the grid point.

An example of velocity estimates obtained assuming the simple fluid approximation is shown in Fig. 2. Both STARE and SABRE data are included with the SABRE field of view located to the south-east. A grid point is marked by a dot, and the associated line represents the magnitude and direction of the drift velocity. In this case, the velocities are directed between east and north-east magnitudes up to nearly $1,000 \text{ m s}^{-1}$. To avoid crowding the figure we have only plotted every second grid point both in latitude and in longitude, such that only 25% of the available measurements are shown.

As an example of the SABRE observations we have analysed a Pc5 resonance pulsation event. Such events have been studied extensively with the STARE system¹⁰⁻¹³. Resonance pulsations are thought to have their origin in the Kelvin-Helmholtz instability at the magnetopause. This instability generates a monochromatic evanescent poloidal wave between the ionosphere and the magnetospheric boundary. This wave will resonate with the geomagnetic shell whose eigenfrequency is equal to the wave frequency. At this shell a principally toroidal mode oscillation is excited. On day 83 in 1982, a pulsation event was observed with SABRE, which fast Fourier analysis revealed to be monochromatic with a period of 345 s. The east-west velocity component dominates the pulsation, and this component shows no significant variations with longitude. The amplitude and phase of this toroidal component are shown as functions

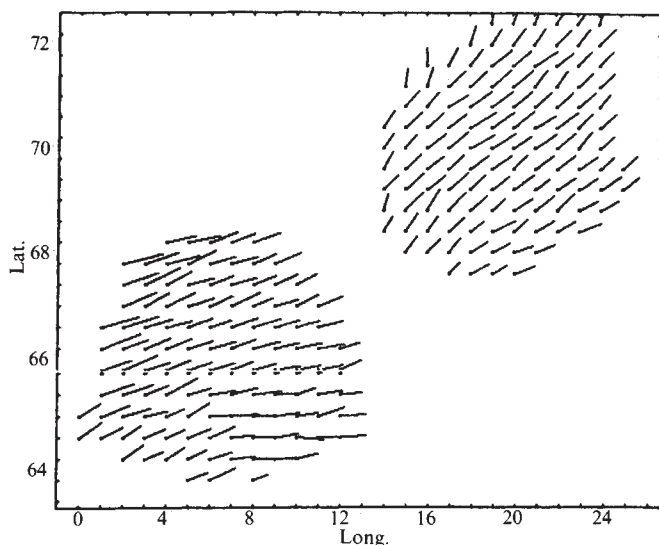


Fig. 2 Example of simultaneous STARE and SABRE observations taken at 014140 UT on day 93 of 1982 in the westward electrojet region. Irregularity drift velocity, $1,000 \text{ m s}^{-1}$.

Table 1 Radar parameters

Gain of broad beam TX antenna	17 dB
Transmitted power	45 kW
TX pulse length	100 μs
Double pulse separation	300 μs
Gain of RX-antenna	28 dB
Number of RX-antenna lobes	16 (8 are used)
Audio amplifier rise time	100 μs
Receiver dynamic range	50 dB
Lag to first sample	3.4 ms
	(= 495 km)
Sample separation	100 μs
	(= 15 km)
No. of samples (ranges) per lobe	50

of latitude in Fig. 3. The curves represent a least squares fit of a simple resonance model to the observations¹³. The good fits indicate that it is a resonance event. The most striking feature of these observations is the low value of the amplitude at resonance, only $\sim 80 \text{ m s}^{-1}$ ($= 4 \text{ mV m}^{-1}$). This is less than half of the lowest value ever observed at higher latitudes with the STARE system. Such a qualitative relationship is to be expected. The amplitude of the evanescent wave, which excites these pulsations, is exponentially decreasing with distance from the magnetopause. If the efficiency, with which the wave couples to the resonating geomagnetic shell, is independent of location (L -value), then generally lower pulsation amplitudes would be expected as the resonance latitude decreases—in agreement with the observations. The width of the amplitude resonance peak is a measure of the energy loss from the driving wave to the ionosphere, where energy is dissipated through Pedersen currents. The observed width is consistent with a Pedersen conductivity of between 0.9 and 1.4 S (refs 14, 15). This is close to model predictions^{16,17} of $\sim 2 \text{ S}$.

The observations of an ultra low frequency-pulsation event characterized by a large azimuthal wavenumber and a large westward directed phase velocity have recently been reported¹⁸, such an event was observed simultaneously with the STARE and SABRE systems and the data analysis of this event has been carried out as described in ref. 18. Figure 4 shows the phase variation of the pulsating east-west velocity component as a function of geomagnetic longitude. The data points in the left-hand side of Fig. 4 are from the SABRE system. The phases observed with the two systems are not normalized. Figure 4 therefore clearly shows that a wave is propagating westward from the STARE field of view to the SABRE field of view.

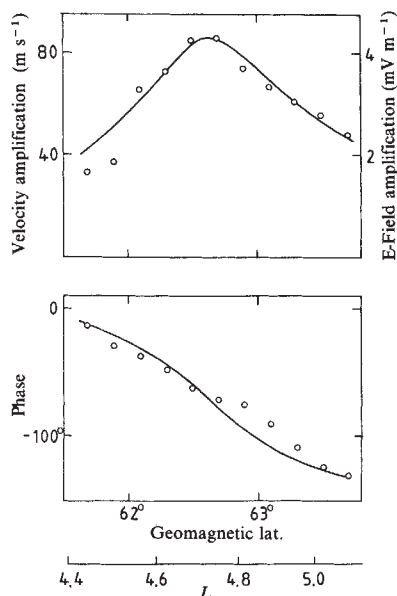


Fig. 3 The amplitude and phase variations of the east-west (toroidal) velocity component of a Pc5 resonance pulsation, observed between 1730 and 1830 UT (2000–2100 MT).

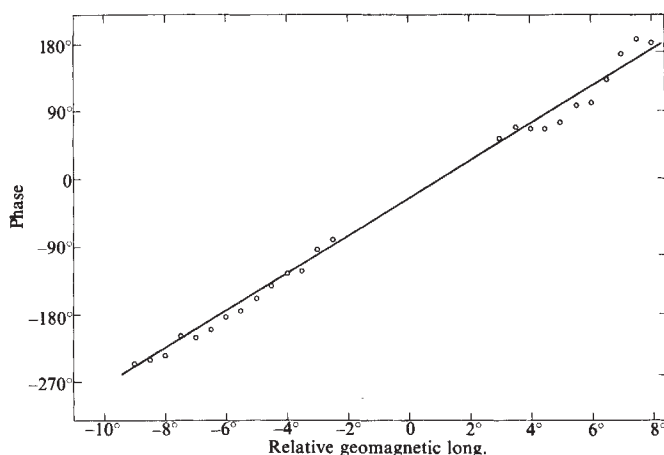


Fig. 4 The phase variation of the east-west velocity component along a constant geomagnetic latitude (66.5°). The linear fit of the data points yield an azimuthal wave number of 26.

Thus, this kind of geomagnetic pulsation can be coherent over at least 17° long., nearly doubling the coherence interval derived from the STARE data alone.

These two examples show that the large field of view together with the good spatial and temporal resolution make the combined STARE and SABRE data of unique value for geophysical studies¹⁹.

Received 3 May, accepted 15 June 1983.

1. Sudan, R. N., Akinrimisi, J. & Farley, D. T. *J. geophys. Res.* **7**, 240–248 (1973).
2. Nielsen, E. & Whitehead, J. D. *Proc. COSPAR*, Ottawa (in the press).
3. Schlegel, K. *Radio Sci.* (in the press).
4. Schlegel, K. & St-Maurice, J. P. *J. geophys. Res.* **86**, 1447–1452 (1981).
5. St-Maurice, J. P., Schlegel, K. & Banks, P. M. *J. geophys. Res.* **86**, 1453–1462 (1981).
6. Nielsen, E. & Schlegel, K. *J. geophys. Res.* (in the press).
7. Greenwald, R. A., Weiss, W., Nielsen, E. & Thomson, N. *Radio Sci.* **13**, 1021–1039 (1978).
8. Redfern, J. *Nature* **290**, 727 (1981).
9. Rummel, W. D. *MM-68-4121-15* (Bell Telephone Laboratory, New York, 1968).
10. Walker, A. D. M., Greenwald, R. A., Stuart, W. F. & Green, C. A. *J. geophys. Res.* **84**, 3373 (1979).
11. Poulter, E. M. *J. geophys. Res.* **87**, 8167–8173 (1982).
12. Poulter, E. M., Nielsen, E. & Potemra, T. *J. geophys. Res.* **87**, 2331–2336 (1982).
13. Nielsen, E. & Allan, W. *J. geophys. Res.* (in the press).
14. Allan, W. & Knox, F. B. *Planet. Space Sci.* **27**, 79–85 (1979).
15. Allan, W. & Knox, F. B. *Planet. Space Sci.* **27**, 939–950 (1979).
16. Spiro, R. W., Reiff, P. H. & Maher, L. J. Jr *J. geophys. Res.* **87**, 8215–8227 (1982).
17. Wallis, D. D. & Budzinski, E. E. *J. geophys. Res.* **86**, 125–137 (1982).
18. Allan, W., Poulter, E. M. & Nielsen, E. *J. geophys. Res.* **87**, 6163–6172 (1982).
19. Nielsen, E. *The IMS Source Book* (eds Russell, C. T. & Southwood, D. J.) 213–224 (American Geophysical Union, Washington, DC, 1982).

Effects of glacial transport and neomorphism on Precambrian dolomite crystal sizes

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Late Precambrian glacial rocks are notable for their association with dolostones¹ and this has been regarded as a palaeoclimatological paradox^{2–5}. However, little detailed work has been carried out to determine the mode of origin of the dolomite. In north-east Spitsbergen (Svalbard), the Vendian^{6,7} succession contains two glacial stratigraphical units^{8,9} which are dolomitic and are underlain and overlain by dolostones. Evidence from sedimentary structures and gross textures indicates that dolomite within the glacial units is detrital whereas at other levels it is a precipitate¹⁰. Accordingly, dolomite crystal sizes were analysed to determine whether evidence of glacial erosion and transport (rock flour) has been preserved. Allowing for the effects of later crystal enlargement (aggrading neomorphism), which vary within and between samples, the results show a markedly higher proportion of very fine crystals in the inferred detrital dolostones compared with the precipitated dolostones. The very fine crystals are interpreted as relics of rock flour, the preservation of which demonstrates a novel means of testing the origin of dolostones associated with glacial sequences. Inorganic precipitation of dolomite during glaciation or secondary dolomitization of till are not required to explain the dolomite-tillite association, but pronounced climatic changes at the onset and close of glacial periods are indicated.

The Vendian sequence of north-east Spitsbergen, unmetamorphosed and lacking penetrative deformation, contains diamictite-bearing stratigraphical units whose glacial origin has been well documented^{8,10–13}. These are the Wilsonbreen Formation^{11–14} and the Petrobreen Member^{8,9} of the Elbobreen Formation^{11–14} (Fig. 1). They comprise largely diamictites (matrix-supported conglomerates) with subordinate conglomerates, sandstones, rhythmites and poorly laminated sediments with dropstones, all of which are dolomitic. Clasts in the Petrobreen diamictites are overwhelmingly dolostones like those of the basal Elbobreen Formation whilst the Wilsonbreen Formation contains a mixture of extrabasinal fragments and dolostones like those of the underlying formations. The dolomitic nature of the diamictite matrix, which is particularly pronounced in the Petrobreen Member, can therefore be inferred to be due to comminution of dolostone fragments.

The glacial units are underlain and overlain by finely crystalline dolostones with subordinate clay, and silt-grade quartz and feldspar. An origin of the carbonate by precipitation in the depositional environment is indicated by the presence of ooids, peloids, uniform finely crystalline dolomite layers with derived intraclasts, and evidence of elevated salinities (anhydrite inclusions in quartz nodules). Proof of an early stage of dolomite formation is a separate argument because the dolomite may have replaced another carbonate. It is indicated by the excellent textural preservation as is common in other Precambrian dolomites^{15,16}: finely crystalline allochems are bounded by fibrous (Fig. 3a) or equant sparry (up to 50 μ m) dolomite mosaics that are clearly cements or replacements of cements. In the diamictites, these cement crystals are truncated at the margins of fragments so that the source materials for the (crushed) diamictite matrix would have consisted of dolomite mosaics up to 50 μ m in crystal size.

Having made these deductions from field study and normal-petrographic analysis of 230 specimens, 10 samples were selected for crystal size analysis in order to compare the precipitated dolomite with the inferred products of glacial erosion and

transport of such dolomites. This was carried out on ultrathin (2–4 μm) sections¹⁷. Photographs were taken in plane-polarized light and in two different orientations using crossed polars. Crystal boundaries were traced on $\times 2,000$ enlargements and corrected by direct microscopic observation of the thin section. The long axis of each crystal was measured and size distributions computed. No other comparable studies on dolomite are evident in the literature.

Results for precipitated dolomites are shown in Fig. 2a–e. These samples were selected as representative of the most finely crystalline dolomite from the non-glacial stratigraphical units. Median size ranges from 3.3 to 7.5 μm with 0–5% of crystals $< 1 \mu\text{m}$ in size. Crystals are dominantly polygonal rather than irregular in shape but boundaries are frequently curved rather than straight (Fig. 3a). These characteristics are similar to many calcite micrites (reviewed in ref. 18) although some micrites are rather finer. Modern carbonate muds are typically finer¹⁸ and even replacive dolomite mosaics can be sub-micrometre-sized¹⁹ so that it is highly likely that the mosaics of Fig. 2a–e are coarser than they were during formation of the glacial units. Despite this complicating factor, the paucity or absence of sub-micrometre-sized crystals is distinctive.

The dolomite of the diamictite of Figs 2f and 3b is quite different as here the mode is now in the sub-micrometre size class. (An ultrathin section of an unusually dolomite-rich Wilsonbreen Formation diamictite appears identical optically but its size distribution was not measured.) This sub-micrometre sized mode is thought to represent the remnant of rock flour produced during glacial erosion and transport. Much secondary loss of very fine crystals originally present would have occurred because such crystals, especially having been mechanically stressed, would have substantial excess free energy. Note that most of the sub-micrometre crystals now present are euhedral rhombs, indicative of secondary enlargement. The size distribution cannot be taken to prove glacial rather than another mode of transport as there is no comparable information on detrital dolomite in diamictites of non-glacial origin. However, given the glacial origin deduced by other criteria, the size distribution could serve as a standard for comparison with diamictites of

uncertain origin. One would expect that dolostone erosion and transport in non-glacial regimes would not lead to large amounts of rock flour production.

The other samples from the glacial units are from non-diamictite lithologies. Figure 2g is from the dolomite matrix of a crudely laminated sandy dolostone with wackestone fabric that appears to have formed by suspension sedimentation of glacial detritus. The distribution is comparable with Fig. 2f.

Figure 2h and 3c derive from a dolostone, lacking primary structure, which is bounded above and below by diamictite beds. The median here is slightly higher at 2.3 μm , which relates to the presence of fewer sub-micrometre sized crystals, but the mosaic is distinctly finer than any of the precipitated dolomites from non-glacial horizons. An origin by uniform sedimentation

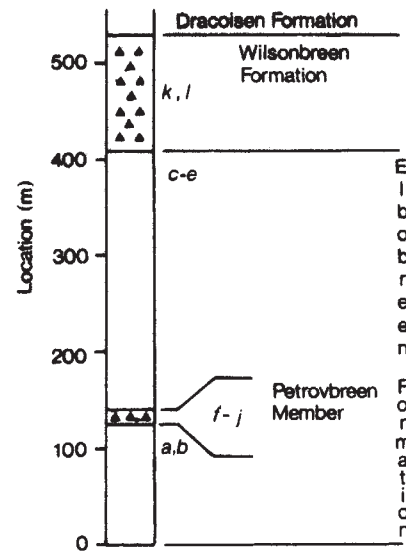


Fig. 1 Stratigraphical location of samples within the Vendian succession (Ditlovtoppen and Dracoisen areas, Ny Friesland region). Letters refer to histograms of Fig. 2.

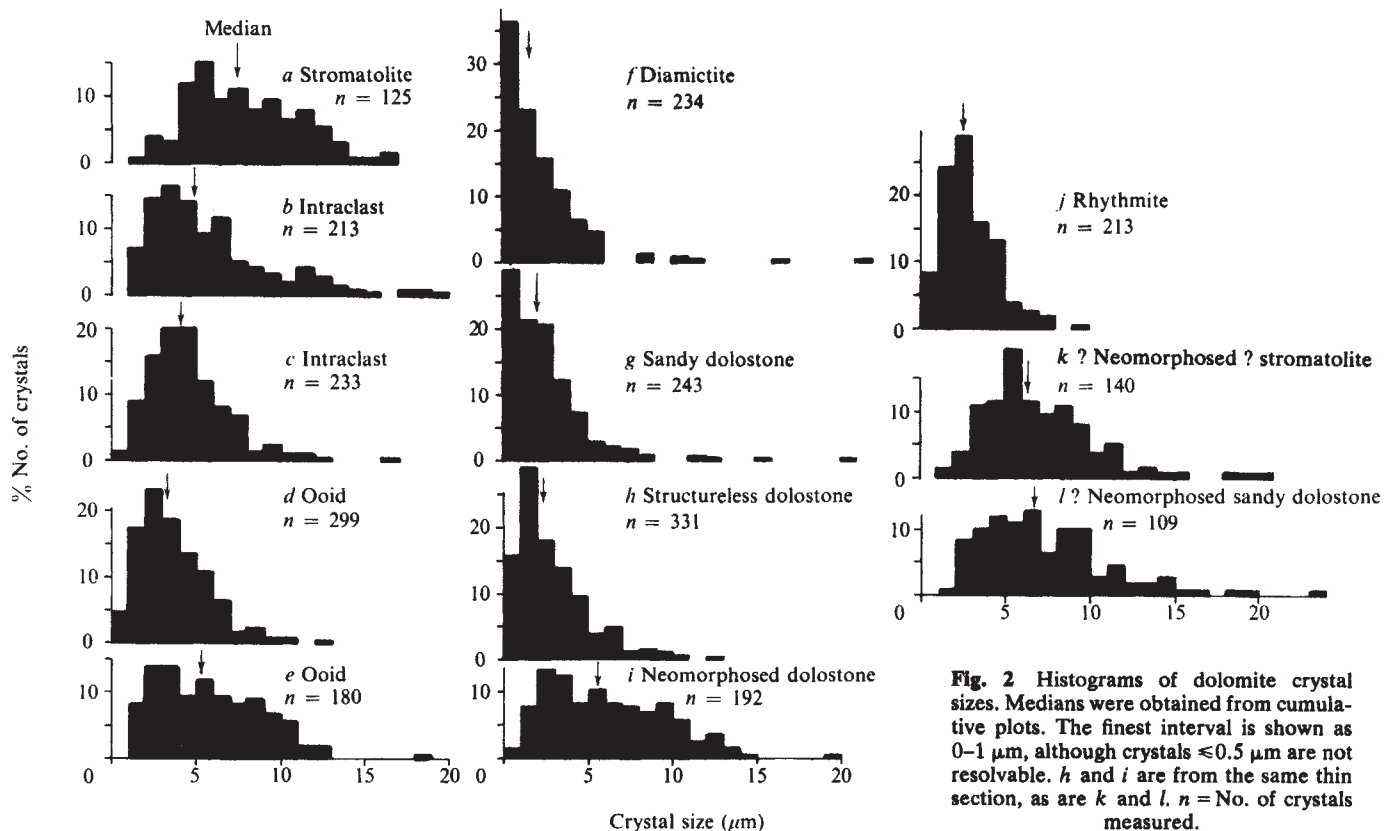


Fig. 2 Histograms of dolomite crystal sizes. Medians were obtained from cumulative plots. The finest interval is shown as 0–1 μm , although crystals $\leq 0.5 \mu\text{m}$ are not resolvable. h and i are from the same thin section, as are k and l. n = No. of crystals measured.

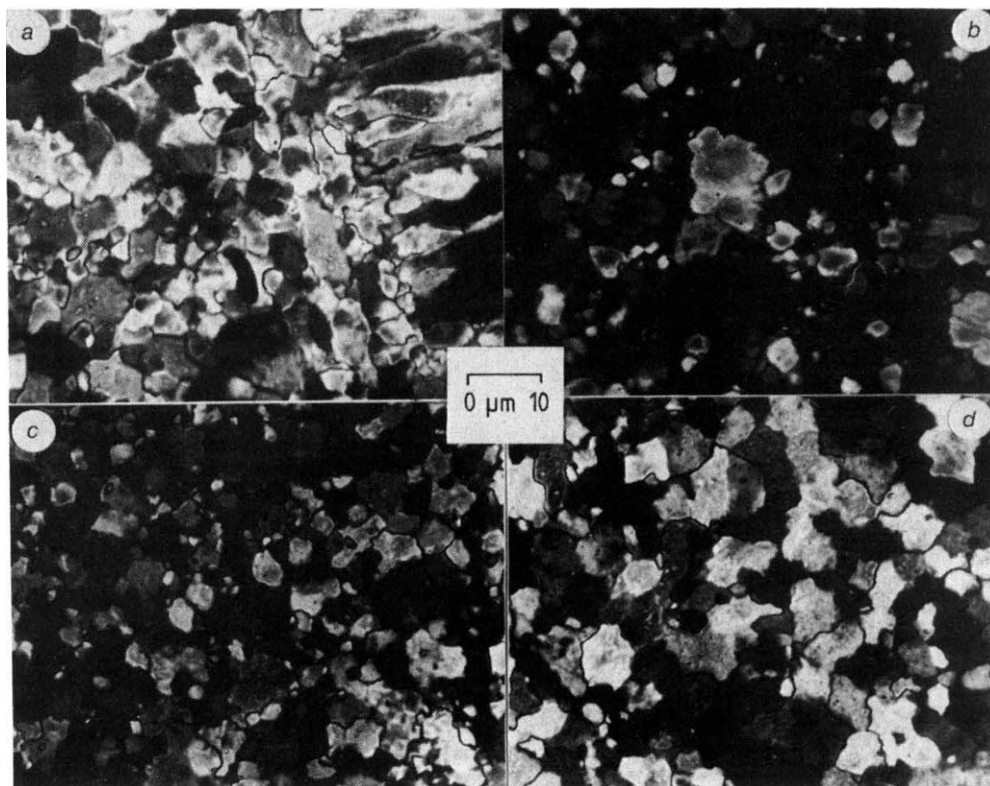


Fig. 3 Photomicrographs of thin sections under crossed polars. *a*, Ooid (Fig. 2*e*) bounded by fibrous dolomite 'cement' on right. *b*, Diamictite (Fig. 2*f*). Most of dark areas are quartz (which appears isotropic in ultrathin sections). Smaller dolomite crystals are rhombic. *c*, Dolostone (Fig. 2*h*) interpreted as forming by suspension sedimentation of glacially transported particles. *d*, Dolostone (Fig. 2*i*) from the same thin section as Fig. 2*h*. Neomorphism has led to the loss of most of the smaller crystals.

from suspension of fine glacial debris is thus proposed. The increase in median size from Fig. 2*f* to 2*g* and 2*h* correlates with a decrease in quartz content. In principle one would expect fine dolomite crystals to be more stable adjacent to quartz rather than dolomite as in the latter case a reduction in free energy is readily attained by the enlargement of a larger crystal, at the expense of a smaller crystal. Figure 2*j* is from a rhythmically laminated dolostone, exhibiting regular 0.3-mm graded layers, occurring in a unit between sandy dolostones like that of Fig. 2*g*. Sedimentation from suspension of glacially transported detritus is indicated by the rhythmic layering. The crystal size analysis supports this, given that the median is distinctly finer than any of the precipitated dolomites, but again one must invoke much loss of very fine crystals by neomorphism of this pure dolomite mosaic.

Figures 2*i* and 3*d* represents an area from the same thin section as Fig. 2*h*, but which is considerably coarser (median of 5.6 μm). Areas like that of Fig. 3*d* form diffuse vein- and net-like regions surrounding areas similar to Fig. 3*c*. Clearly this is a secondary, neomorphic effect and illustrates how evidence for a substantial fine crystal mode can be destroyed.

Figure 2*l* represents a sandy dolostone (occurring within a series of laminated or bedded sediments between diamictite beds) that resembles the rock of Fig. 2*g* in ordinary thin sections, but turns out to be much coarser, and similar in crystal size distribution to a possible stromatolitic layer (Fig. 2*k*) in the same thin section. Thus the crystal size analysis here does not uphold a glacial-detrital origin, although the evidence may have been obscured by neomorphism.

The *a priori* precipitated dolomites therefore show median size greater than 3 μm and are quite distinct from most of the inferred glacial-detrital dolomites with median size well below 3 μm , particularly in less pure dolomite mosaics where neomorphism has been less active. The vagaries of neomorphism (Fig. 3*c* and *d*) can, however, destroy the evidence of a fine mode in crystal size.

Fieldwork was undertaken as a member of the 1982 Cambridge Spitsbergen Expedition supported by a NERC grant (GR3/4342) to expedition leader W. B. Harland. I thank co-worker and expedition organizer M. J. Hambrey for discussion and P. Hands for the ultrathin sections.

Received 12 May; accepted 16 June 1983.

1. Hambrey, M. J. & Harland, W. B. (eds) *Earth's Pre-Pleistocene Glacial Record* (Cambridge University Press, 1981).
2. Spencer, A. M. *Mem. geol. Soc. Lond.* **6** (1971).
3. Schermerhorn, L. J. *G. Am. J. Sci.* **274**, 673–824 (1974).
4. Williams, G. E. *Geol. Mag.* **112**, 441–465 (1975); *J. geol. Soc. Aust.* **26**, 377–386 (1979).
5. Harland, W. B. *J. geol. Soc. Lond.* **138**, 197–203 (1981).
6. Milstein, V. E. & Golovanov, N. P. *Skr. Nor. Polarinst.* **167**, 219–224 (1979).
7. Knoll, A. H. *Geol. Mag.* **119**, 269–79 (1982).
8. Hambrey, M. J. *Geol. Mag.* **119**, 527–551 (1982).
9. Hambrey, M. J. *Geol. Mag.* **120**, 209–232 (1983).
10. Fairchild, I. J. & Hambrey, M. J. *Precambrian Res.* (submitted).
11. Chumakov, N. M. *Doklady SSSR* **180**, 1446–1449 (1968); *Trudy GIN SSSR* **308**, 12–87, (1978).
12. Hambrey, M. J., Harland, W. B. & Waddams, P. *Earth's Pre-Pleistocene Glacial Record* (ed. Harland, W. B.) 592–601 (Cambridge University Press, 1981).
13. Wilson, C. B. & Harland, W. B. *Geol. Mag.* **101**, 198–219 (1964).
14. Harland, W. B., Wallis, R. H. & Gayer, R. A. *Geol. Mag.* **103**, 70–97 (1966).
15. Fairchild, I. J. *J. sedim. Petrol.* **50**, 423–446 (1980).
16. Tucker, M. E. *Geology* **10**, 7–12 (1982).
17. Lindholm, R. C. & Dean, D. A. *J. sedim. Petrol.* **43**, 295–297 (1973).
18. Bathurst, R. G. C. *Carbonate Sediments and their Diagenesis* (Elsevier, Amsterdam, 1975).
19. Sibley, D. F. *J. sedim. Petrol.* **52**, 1087–1100 (1982).

Co-fluxes and growth rates in ferromanganese deposits from central Pacific seamount areas

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Recent geochemical studies on ferromanganese deep-sea nodules have shown that the relationship between Mn and Fe allows us to differentiate between a diagenetic type ($\text{Mn/Fe} > 2.5$) and a hydrogenetic type ($\text{Mn/Fe} < 2.5$)^{1–7}. Diagenetic and the lower parts of mixed type nodules are mainly supplied by an early diagenetic pore water source; hydrogenetic type nodules grow by very slow accumulation of inorganic colloidal particles of hydrated metal oxides from near-bottom seawater. Growth rates of hydrogenetic nodules generally do

not exceed 5 mm Myr^{-1} (refs 8–11), whereas diagenetic nodules were found to grow much faster: an extreme example is the rate of 168 mm Myr^{-1} that was recently reported for a Mn-rich diagenetic type of nodule from the Peru Basin⁶. A different inter-element relationship of Co to the main metals Mn and Fe in different type of nodules has also been observed. We show here that the flux of Co into ferromanganese seamount crusts from the central Pacific is similar to the flux into pelagic nodules: the amount of Co supplied per unit of time and area is nearly constant in the oceanic water column. The most important factor governing the Co-enrichment is the growth rate; no local source is needed to explain the observed Co concentrations up to 2% in ore samples from summit areas.

In early diagenetic and mixed type nodules Co displays a positive correlation with Fe ($r = 0.77$)³ and no correlation to Mn, whereas in hydrogenetic nodules Co correlates to Mn ($r = 0.79$)³. Hydrogenetic nodules and crusts show generally higher Co contents than diagenetically formed nodules. This observation in relation to the variability of the growth rates suggests that the Co-enrichment in crusts and nodules should be inversely related to the growth rate: the slower the growth rate the higher the Co concentration. The higher Co contents in ferromanganese deposits from shallower seamount areas have, however, alternatively been explained as being the result of local supply of Co (ref. 12). Two arguments seem to support this idea: (1) the lack of sediment blanket, insulating the volcanic rocks from weathering in seawater, and (2) the fact that the basaltic lava from subaerial or shallow submarine volcanism shows a highly vesicular structure which might result in a more intense submarine weathering¹³.

^{230}Th and ^{231}Pa datings on crust samples with a high Co content (0.5–2%) from the Line Islands Ridge and the Mid-Pacific Mountains that were recently taken during the Midpac 81 cruise¹⁴, enable us to test these contradictory models. This is mainly achieved by comparing Co-fluxes into six ferromanganese crusts from different water depths and their Co concentrations, with data derived from deep-sea nodules of hydrogenetic origin from other areas in the north-east Pacific. The metals found in the seamount Mn-crusts can only have

seawater as possible source: an immediate hydrothermal supply can be excluded because the basaltic volcanism that produced the basement rocks of the Line Islands Ridge and the Mid-Pacific Mountains is older than around 70 Myr BP, and the deposits, containing a main mineral phase of δMnO_2 intimately intergrown with X-ray amorphous $\text{FeOOH} \cdot \text{H}_2\text{O}$ (ref. 15), have been precipitated immediately on the solid substrate rocks without an intervening sediment layer. On seamount plateaus, nodules are distributed on partly consolidated calcareous ooze¹⁴. The plateau nodules contain mostly nuclei of altered rock fragments, therefore, their bulk composition shows lower metal concentrations. Indeed, they were formed by a hydro-geogenic precipitation process similar to that in the seamount crusts.

The metal composition of the outermost millimetre of the crusts (where growth rates were determined) is given in Table 1. We observe an increase in metal concentration with decreasing water depth: Co increases above the carbonate compensation depth (CCD in Fig. 1) from <0.4% at depths >4,000 m to >1% in seamount regions of <2,000 m water depth. Maximum Co concentrations (up to 2%) are found in the outer 0.5 cm of crusts from depths between 1,500 and 1,100 m. Mn and Ni are positively correlated with Co ($r = 0.79$; $r = 0.54$; $n = 46$)¹⁴, whereas Cu shows an inverse relationship to these metals, which is in contrast to the situation in typical north-east Pacific deep-sea nodules⁷. The samples are characterized by low Mn/Fe ratios (Table 1). Within the group of hydrogenetic precipitates the ferromanganese encrustations from seamount areas with shallow water depth show higher Mn/Fe ratios than hydrogenetic nodules from deeper water areas. A distinct increase in the trend of the Ni and Co gradient exists in water depths of around 2,000 m (Fig. 1). This is unlike the situation beneath the CCD where Co displays a further decrease with water depth towards values of 0.3%, Ni shows an increase up to values of ~0.6% (Fig. 1); however, both trends are not very significant as the individual values show large variations. Typical deep-sea nodules from the north-east Pacific contain Ni concentrations distinctly higher than 0.6% and Co concentrations below 0.3% (refs 4, 7) which confirms these metal trends

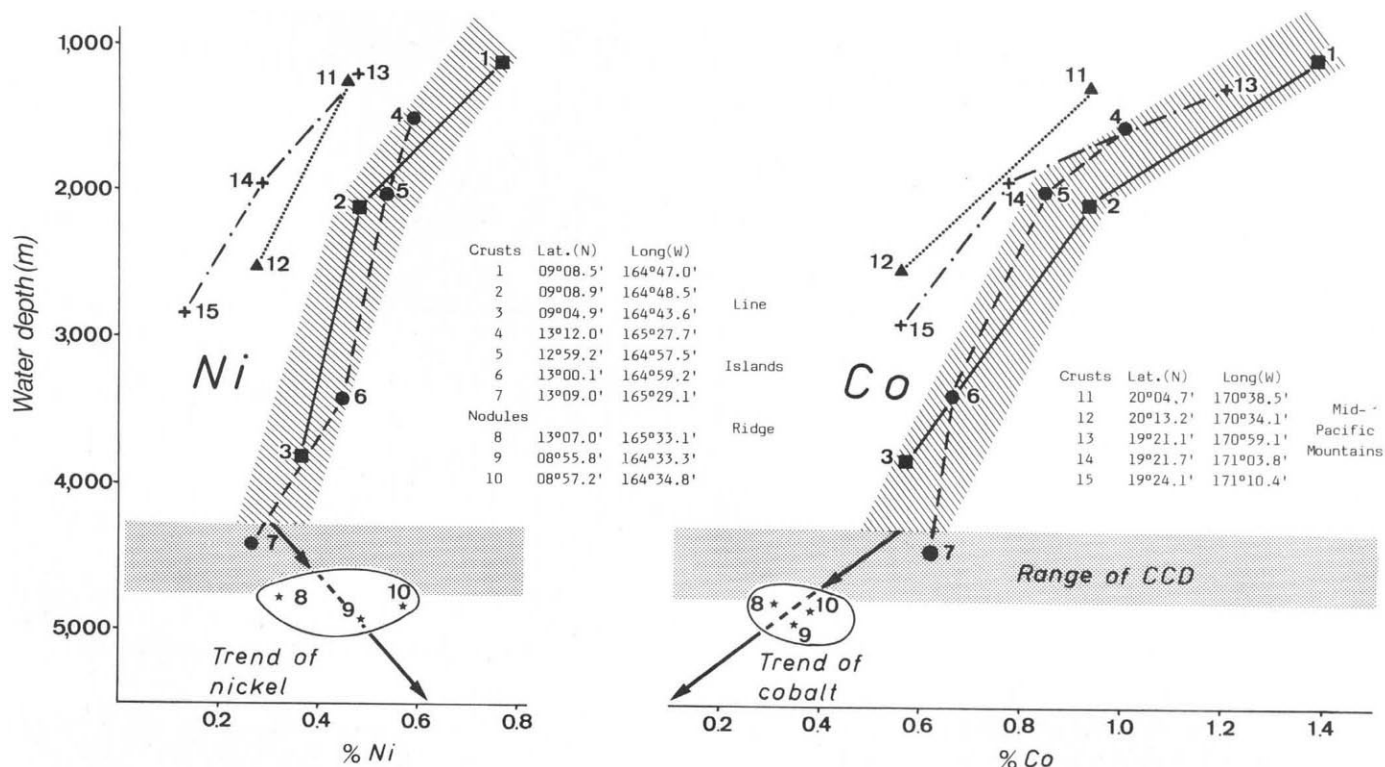


Fig. 1 Concentration of Co and Ni in ferromanganese crust and nodule samples (Midpac 81) versus water depth; numbers refer to sample sites. The plotted values were obtained by the calculation of mean values from dredge stations.

Table 1 Metal composition (wt % of dried substance), water depth and location of the investigated seamount samples

Sample	Mn	Fe	Ni	Co	Cu	Mn/Fe	Water depth (m)	Long. (W)	Lat. (N)
57 DK 2	21.4	19.0	0.27	0.62	0.10	1.13	3,280	165°29.1'	13°09.0'
111 DK 2	26.7	14.6	0.48	1.30	0.02	1.83	1,240	170°38.5'	20°04.7'
76 DK	25.7	16.7	0.47	0.91	0.04	1.54	1,190	170°59.0'	19°21.1'
73 DK 7	19.9	17.5	0.25	0.57	0.12	1.14	2,860	171°10.4'	09°24.1'
32 DK 2	31.4	9.5	0.62	2.0	0.05	3.31	1,120	164°47.0'	09°08.5'
31 DK 5	23.7	14.5	0.38	0.78	0.05	1.63	2,100	164°48.5'	09°08.9'

Mn, Fe were determined by potentiometric and complexometric titration methods; Ni, Cu, Co were analysed using differential pulse polarography. All analytical data are results of repeated determinations having <1% of relative error for main constituents (Mn, Fe), and <5% of relative error for minor constituents (Ni, Co, Cu).

Table 2 Growth rates and Co-, Mn-, and Ni-fluxes into investigated crusts and Mn-nodules with relatively low Mn/Fe ratios

Sample	Growth rate (mm Myr ⁻¹)	Co-flux (μg cm ⁻² kyr ⁻¹)	Mn-flux (μg cm ⁻² kyr ⁻¹)	Ni-flux (μg cm ⁻² kyr ⁻¹)	Ref.
Crusts					
57 DK 2	2.7±0.4	2.7±0.44	94±13	1.2±0.18	This work
111 DK 2	1.2±0.06	2.5±0.21	52±5	0.9±0.05	
76 DK	2.7±0.4	4.0±0.73	113±20	2.1±0.31	
73 DK 7	2.6±0.6	2.4±0.4	84±20	1.1±0.15	
32 DK 2	0.8±0.2	2.6±0.4	41±12	0.8±0.20	
31 DK 5	2.7±0.4	3.5±0.5	104±15	1.7±0.25	
Nodules					
KUC 54(2)	9.0±1	2.5±0.3	325±35	12.2±1.95	23
KUC 77	5.5±1.5	3.3±0.9	168±50	8.9±2.44	23
KUC 114(1)	11±1	2.9±0.3	439±50	23.2±2.11	23
Wahine	10.5±0.5	3.1±0.2	420±50		24
Apple	4.7 (top)	2.9–3.5	172–217		25

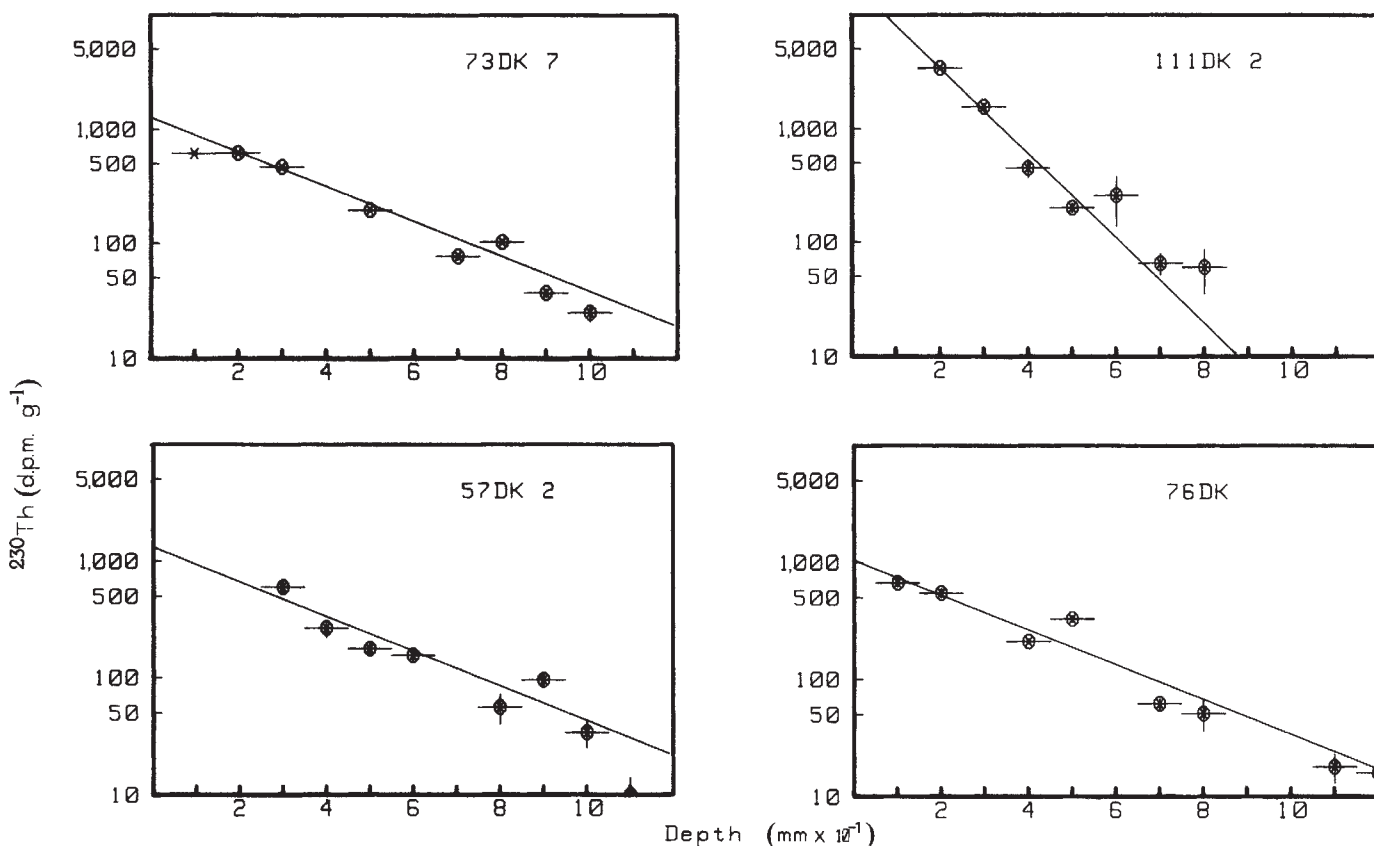


Fig. 2 Plot of $\ln^{230}\text{Th}_{xs}$ against depth for four crusts. The growth rates G (Table 2) were determined from the logarithm of the $^{230}\text{Th}_{xs}$ depth profiles, being given by the inverse slope of the best fit to the data ($-\lambda/G$), where λ is the decay constant of ^{230}Th . A level of 10 d.p.m. g^{-1} was subtracted from total ^{230}Th , corresponding to supported ^{230}Th in equilibrium with ^{234}U , as determined on several samples and being also approximately the value of the lowermost samples in all the studied crusts. The uncertainty range given for G (Table 2) results from the uncertainty in the slope of the weighted fits through the data.

beneath the CCD. Some regional variations of the different metal contents from the Line Islands Ridge samples and the Mid-Pacific Mountains samples, can also be observed (Fig. 1).

The measured growth rates range between 0.8 and 2.7 mm Myr⁻¹ (Table 2). The outermost 1 mm of the crusts was subdivided into 10 samples (each 0.1-mm thick) that were drilled from the surface using a drill bit of 4–8 mm diameter. The Th isotopes were then separated and electroplated applying wet chromatography techniques described elsewhere^{16,17}. To ensure that the derived rates were not an artefact of our sampling techniques, we measured separately a second ²³⁰Th as well ²³¹Pa (through ²²⁷Th) profiles on two crusts (57 DK 2 and 32 DK 2) which yielded consistent results. The ²³⁰Th profiles of four crusts are shown in Fig. 2. The Co- and Mn-fluxes into the crusts (Table 2) were derived as

$$F = C_e G D (\mu\text{g cm}^{-2} \text{ kyr}^{-1})$$

where C_e is the element concentration at the surface, G , the growth rate (mm Myr⁻¹), D , the *in situ* density (1.6 g cm⁻³) (D being derived as γ solid (1- ϵ), where γ solid is the average bulk density of the crust samples (3.25 g cm⁻³) and ϵ the average porosity (0.51))²³.

As shown in Fig. 3, Mn and Co definitely behave differently: the Mn-flux is proportional to the growth rate, in contrast to the Co-flux which appears to be constant over one order of magnitude of growth rate, with an average value of 2.95 $\mu\text{g cm}^{-2} \text{ kyr}^{-1}$. This directly implies that the Co concentration in hydrogenetic ferromanganese deposits must be inversely related to the growth rate. The proportionality between Mn and the growth rate is a trivial one, as Mn is the main component of the precipitate. In the case of the three deep-water nodule samples (Table 2: KUC 54, KUC 114, Wahine), the Mn-flux rises essentially as the growth rate increases. This can be explained by Mn having another source: much of the nodule growth having also been supplied by a diagenetic Mn origin, particularly when those nodules have been formed in sedimentary deep-sea basins.

Similar to Mn and in contrast to Co, the Ni-flux (Table 2) also displays a proportionality to the growth rate, suggesting (1) that Mn is probably the carrier phase for Ni and (2) that the Ni-flux into nodules is not controlled by the amount of Ni available in the water column, as seems to be the case for Co.

The relationship between the metal concentration and the water depth and the lack of sedimentation caused, for example, by the current velocity of the Antarctic bottom water, suggests that the proportionally small rate of calcite dissolution in the water column (for example, 30 $\mu\text{g cm}^{-2} \text{ yr}^{-1}$ from 1,000 to 2,500 m water depth¹⁸) probably controls the metal composition and growth rates of hydrogenetic precipitates in seamount areas. Recent analyses of cleaned calcite skeletons from the Midpac 81 areas¹⁹ have shown that the Fe content is particularly high (400–500 p.p.m. Fe) and is mainly present as amorphous iron hydroxide. Therefore, an increasing supply of iron hydroxide from calcite dissolution might have a diluting effect on Co, Ni and Mn, that is the concentration of these metals should decrease with increasing water depth down to the CCD. The Fe-flux derived from the calcite dissolution rate¹⁹ ($C_{\text{Fe}} = 500$ p.p.m.; $F_{\text{Fe}} = 15 \mu\text{g cm}^{-2} \text{ kyr}^{-1}$) is comparable to that in our seamount crust samples (for example, $C_{\text{Fe}} = 13\%$; $G = 2$ mm Myr⁻¹; $D = 1.6$ g cm⁻³; $F_{\text{Fe}} = 41.6 \mu\text{g cm}^{-2} \text{ kyr}^{-1}$).

Co-fluxes are not different at deeper or shallower water depths, that is the amounts of metal supplied per unit of time and area is nearly constant in the oceanic water column. The most probable mechanism for the ultimate removal of Co from the water column might be a surface reaction of Co with δMnO_2 in the colloidal inorganic particulates. δMnO_2 is specifically able to absorb hydrous Co^{2+} and Ni^{2+} ions. Because of the surface enrichment of Co and the strong electrical field of the Mn(IV), a subsequent oxidation of Co^{2+} to Co^{3+} takes place^{20,21}, leading to the higher enrichment of Co in comparison to Ni. This specific scavenging process of Co might be the reason for the extremely low Co concentration in a seawater depth of

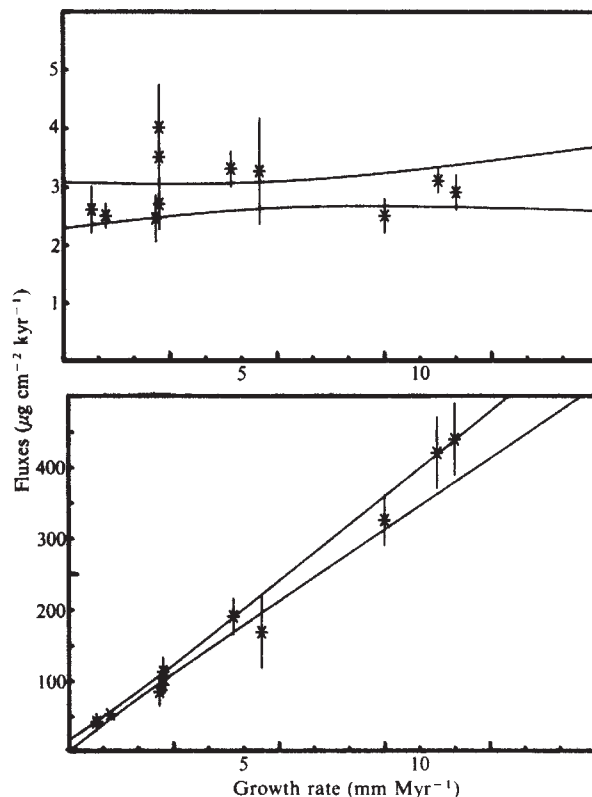


Fig. 3 Plot of the fluxes of Mn and Co against the growth rate on the studied crusts and Mn-nodules described in literature.

more than 700 m (1.4–2.0 ng l⁻¹)²². On the other hand, the concentration of iron in the inorganic particles might have a diluting effect. Indeed, the Mn/Fe ratio shows a positive relationship to Co and Ni in water depth above the CCD.

We conclude that the best pre-conditions for obtaining a high Co concentration in hydrogenetic precipitates, which show in general lower Mn/Fe ratios than early diagenetic and mixed type nodules, are high Mn and low Fe concentrations along with extremely low growth rates, since δMnO_2 is the carrier phase for Co.

Our finding rules out the necessity that Co-enrichment is due to local sources. Moreover, it suggests that the Co content may become a fast and reliable tool for estimating the growth rate of ferromanganese precipitates of hydrogenetic origin.

This research was supported by Deutsche Forschungsgemeinschaft grants Ha 563/21-1 and Mu 199/52-1.

Received 16 December 1982; accepted 28 June 1983.

- Price, N. B. & Calvert, S. E. *Mar. Geol.* **9**, 145–171 (1970).
- Lyle, M., Dymond, J. & Heath, G. R. *Earth planet. Sci. Lett.* **35**, 55–64 (1977).
- Calvert, S. E. & Price, N. B. *Mar. Chem.* **5**, 43–74 (1977).
- Halbach, P. & Özkara, M. *Colloques int. Cent. natn. Rech. scient.* **289**, 77–88 (1979).
- Halbach, P., Hebisch, U. & Scherhag, C. *Chem. Geol.* **34**, 3–17 (1981).
- Reys, J. L., Marchig, V. & Ku, T. L. *Nature* **295**, 401–403 (1982).
- Halbach, P., Scherhag, C., Hebisch, U. & Marchig, V. *Miner. Dep.* **16**, 59–84 (1981).
- Goldberg, E. D. in *Oceanography*, 583–597 (AAAS, Washington DC, 1961).
- Ku, T. C. & Broecker, W. S. *Deep-Sea Res.* **16**, 625–637 (1969).
- Krishnaswami, S. *et al. Earth planet. Sci. Lett.* **59**, 217–234 (1982).
- Heye, D. & Marchig, V. *Mar. Geol.* **23**, M19–M25 (1977).
- Arrhenius, G. *et al. Colloques int. Cent. natn. Rech. scient.* **289**, 333–356 (1979).
- Menard, H. W. *Marine Geology of the Pacific* (McGraw-Hill, New York, 1964).
- Halbach, P. in *Marine Mineral Deposits, New Research Results and Economic Prospects*, 60–85 (Glückauf, Essen, 1982).
- Burns, R. G. & Burns, V. M. *Nature* **255**, 130–131 (1975).
- Krishnaswami, S. & Cochran, J. K. *Earth planet. Sci. Lett.* **40**, 45–62 (1978).
- Mengini, A. & Sonntag, C. *Earth planet. Sci. Lett.* **37**, 251–256 (1977).
- Peterson, M. N. A. *Science* **154**, 1542–1544 (1966).
- Halbach, P. & Puteanus, D. *DFG-Zwischenbericht zum Forschungsvorhaben Ha 563/21-1* (Clausthal-Zellerfeld, 1983).
- Murray, J. W. *Geochim. cosmochim. Acta* **39**, 635–647 (1975).
- Burns, R. G. *Geochim. cosmochim. Acta* **40**, 95–102 (1976).
- Knauer, G. A., Martin, J. H. & Gordon, R. M. *Nature* **297**, 49–51 (1982).
- Halbach, P., Özkara, M. & Hense, J. *Miner. Dep.* **10**, 397–411 (1975).
- Somayajulu, B. L. K., Heath, G. R., Moore, T. C. & Cronan, D. S. *Geochim. cosmochim. Acta* **35**, 621–624 (1971).
- Moore, W. S. *et al. Earth planet. Sci. Lett.* **52**, 151–171 (1981).

140,000-yr isotope climatic record from raised coral reefs in New Guinea

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Darwin¹ was first to draw attention to the unique set of climatic data offered by coral reefs that are preserved on tectonically-active coasts. Geologists have since endeavoured to apply a variety of stratigraphic and radiometric dating techniques²⁻⁴ during their palaeoclimatic investigations of Quaternary coral reefs but the utilization of stable oxygen isotope ratios, so ubiquitous in deep-sea core studies, has not been fully exploited^{5,6}. I report here the results of an oxygen isotope abundance study of the giant clam *Tridacna gigas* from a sequence of uplifted coral reefs in New Guinea which provides a detailed record of changes over the past 10⁵ yr in the surface ocean isotope chemistry and temperature. The combined utilization of sea level and ¹⁸O/¹⁶O data allows a quantitative evaluation of ice volume and temperature factors. The results also contribute to resolving the question as to the exact time-relationship between temperature change at low latitudes and ice accumulation at the latitudes of the glacial ice caps.

The oxygen isotope record and supporting geological and sea level data are shown in Fig. 1. Before discussing the new record in Fig. 1c, the evidence on which it is constructed must be reviewed. The lower sequence of raised coral-reef terraces fringing the north-east coast of Huon Peninsula (6°05' S, 147°36' E) includes seven coral-reef complexes which are best preserved in the area near Sialum⁷ (Fig. 1a). The terraces dated by radiometric techniques^{3,8-10} are spaced between the postglacial reef I (8–6.5 kyr) and the last interglacial interval represented by coral-reef complexes VIIa and VIIb (133 and 120 kyr respectively). The terraces in between (VI, V, IV, III, II dated at 107, 85, 60, 51, to 37, and 28.5 kyr respectively) provide relatively closely spaced 'stratigraphic windows' into the past 10⁵ yr and may be time-equivalents of known episodes of the Laurentide ice partial retreats (ice-age interstadials¹¹).

The difference between the present altitude of an emergent coral-reef of known age, and its estimated tectonic uplift, provides a detailed record of sea-level changes (Fig. 1b). Previous studies support the proposed association of reef building episodes in New Guinea with worldwide glacio-eustatic oscillations of sea level, superimposed on an emerging tectonic landmass^{12,13}.

Oxygen isotope data from each reef phase are tabulated in Table 1 together with estimated sea level and mean age data. Results are based solely on the giant clam species *Tridacna gigas*, rather than corals, for the reasons given in ref. 10. Laboratory procedures for stable isotope analysis are described in detail elsewhere^{10,14}. ¹⁸O/¹⁶O ratios are expressed by δ notation as ‰ difference from a reference standard¹⁵. Over the temperature range of the Indo-Pacific reefs (22–28 °C) the relationship between temperature (T) and the isotopic difference between giant-clam aragonite and water ($\delta T - \delta w$) is well described¹⁴ by the linear equation: T (°C) = 21.30 – 4.42 ($\delta^{18}O_t - \delta^{18}O_w$) without the quadratic term incorporated in Epstein *et al.*'s palaeotemperature equation¹⁶. Multiple giant clams (number as shown in Table 1) were sampled from each coral-reef complex from three cross-sections¹⁴ taken normal to the coast up to coral-reef complex VII (Fig. 1a). All giant clams grew in reef sites exposed to the open ocean either in fore-reef or shallow reef-crest positions. The only exceptions are the

samples from the lagoon VIIb, and the modern lagoon at Y and X, respectively, in Fig. 1a. Pooling the lagoon reefs with the barrier and fringing reefs on the sequence is justified by (1) the detailed structure of the raised lagoons that is similar to the modern ones fringing the Huon coast where the waters are well circulated¹⁰; (2) samples from the modern lagoon and fringing reef sites that show identical isotopic compositions within the experimental error of $\pm 0.10\%$ (ref. 14).

The $\delta^{18}O$ record in Fig. 1c shows that a series of isotopic variations occurred during the transition period from the last interglacial to the present interglacial. These variations are superimposed on a general trend of ¹⁸O enrichment of 0.7‰ between the earlier high sea level phases (reefs VI, V) and the later phases (reefs IV, III, II). The ¹⁸O enrichment reached a maximum during a regressive phase at ~43 kyr when $\delta^{18}O$ differs by 1.75‰ from present values. A reversed trend of ¹⁸O depletion during the early postglacial phase I, and a return to about the previous interglacial levels during growth of the modern reef, completes the climatic cycle.

The coral-reef isotope record is compared in Fig. 2 with $\delta^{18}O$ curves from two apparently undisturbed deep-sea cores that probe the tropical ocean surface^{17,18}. The results compare closely on time scales ranging from 10⁵ to 10⁴ yr. Inconsistencies observed on shorter time scales have been shown to reflect differences in the resolving power of the individual records^{10,14} and the complex regional dynamics of the different ocean basins¹⁸. The conspicuous marine records, however, raise the question of their climatic significance. Concerning the isotope variations, two climatic variables leave a quantitative mark on the ¹⁸O/¹⁶O ratios of marine carbonates in well-mixed ocean waters. A temperature change of 1 °C affects the degree of ¹⁸O-enrichment in skeletal CaCO₃ relative to seawater by ~0.23 ‰. A transfer of $\sim 3.6 \times 10^6$ km³ water in the ocean-cryosphere system causes 10 m of sea-level change; a transfer equivalent to h m affects $\delta^{18}O$ by $(h/3,800)(\delta^{18}O_{ice} - \delta^{18}O_{ocean})$. The latter effect depends on the extent of ¹⁸O-deficiency in the continental ice relative to ocean which is likely to have varied significantly between distinct climatic modes¹⁹.

Interpretations of the $\delta^{18}O$ variations in the tropical deep-sea cores shown in Fig. 2 led to divergent estimates of ice volumes and temperature changes for time-equivalent events. For example, Emiliani¹⁷ concluded that ~70% of the observed variations can be ascribed to the temperature effect and the remainder to ice-volume effect. More recently, Shackleton and Opdyke¹⁸ have argued that the latter is the predominant factor in the tropical Pacific cores. The two extreme views use the glaciation maximum spike at 18 kyr as their yardstick and assume a constant ratio of the climatic variables beyond this reference point. In the coral-reef record, by contrast, the ice volume factor can be objectively assessed for each reef phase and decoupled from the temperature effect on the $\delta^{18}O$ compositions, on the premise that: (1) sea-level data are a first-order function of ice volume changes; and (2) $\delta^{18}O$ measurements of ice cores in Greenland and Antarctica²⁰ indicate the general temporal pattern of ¹⁸O-depletion of the glacial ice and provide average values of –37‰ and –31‰ during extensive ice periods (70–18 kyr) and warmer conditions (100–70 kyr) respectively²¹.

The effect of continental glacial ice-volume is decoupled from temperature in Table 1 as follows. An isotope sea level is calculated for each reef phase by assuming that the difference in the $\delta^{18}O$ composition between any reef and the modern derives solely from the transfer of ¹⁸O-depleted waters to the ice caps. Should the isotope sea level be significantly different, statistically speaking, from the stratigraphic sea level previously established for that reef phase, then the $\delta^{18}O$ residual either is taken as being temperature related, or must be attributed to excess non-continental glacial ice²². The latter will be registered by $\delta^{18}O$ -data but not by sea-level data because marine-based ice displaces 90% of its own volume of water.

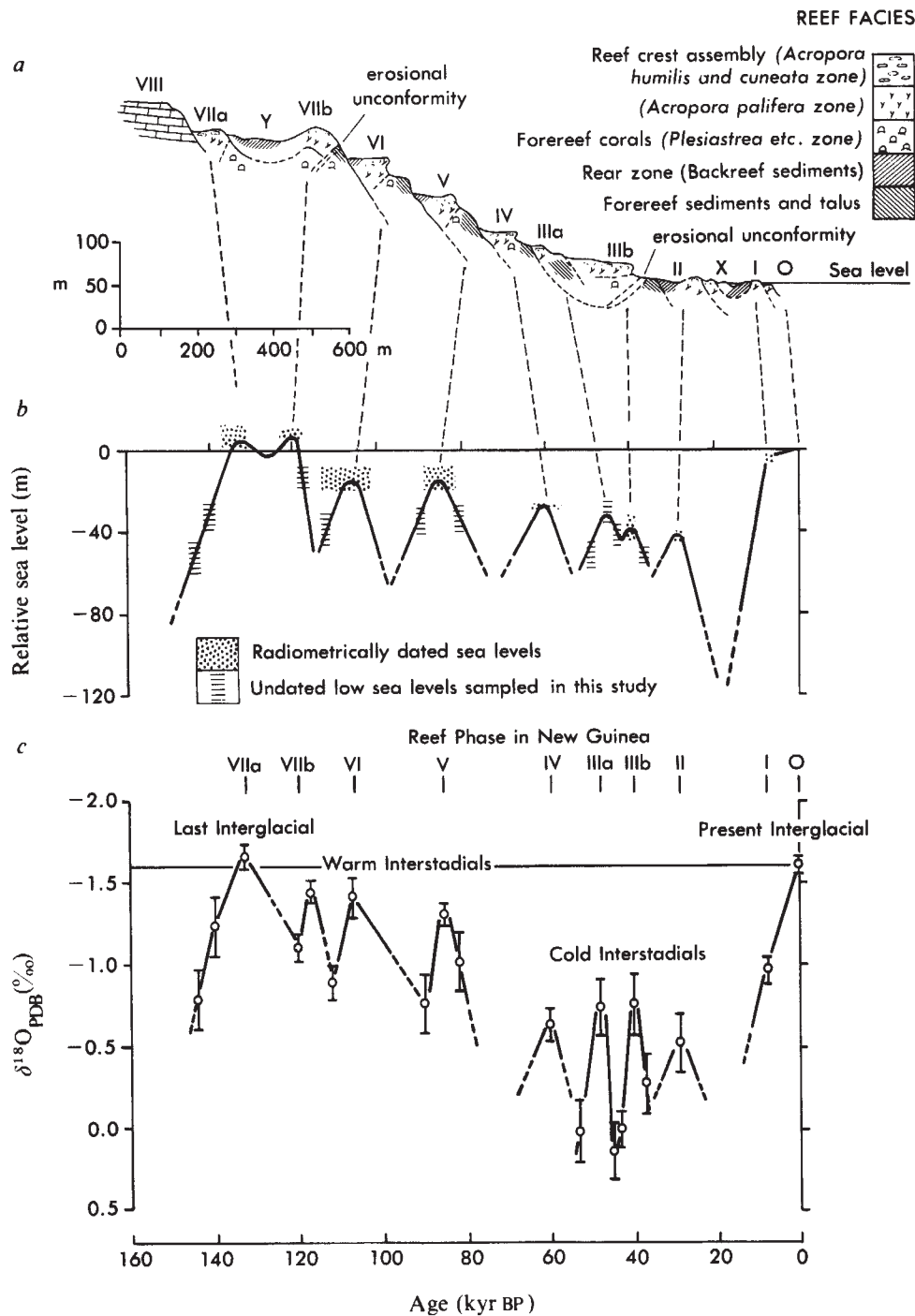


Fig. 1 Reconstructed $\delta^{18}\text{O}$ record for the past 10^5 yr and supporting geological and sea-level data. *a*, Cross-section of New Guinea reefs at Sialum. *b*, Sea levels according to refs 3, 14. *c*, $\delta^{18}\text{O}$ record. Small circles are group means for each reef facies. Bars represent the pooled standard error ($1\sigma_x$) estimate (see Table 1).

The interpretation of the isotopic residuals in Table 1 as indicating substantial surface temperature changes during late stages of the ice age is supported by: (1) evidence based on factor analysis counts of coccoliths from deep-sea core V28-238 (ref. 23) that indicates episodic cooling of up to 5°C below present during isotope stage 3 (both the Huon coral reefs and the sediments of core V28-238 pertain to the same oceanographic province of the westward equatorial current flow)¹⁴; (2) relatively rapid fluctuations between 51 and 37 kyr of magnitude and rate that seem too great to be attributable to ice volume changes; and (3) early Holocene results which are from a period (7 kyr) when global ice volumes and sea level were close to present conditions. Hence the alternative proposed by Williams *et al.*²⁴ that the isotopic residuals between $\delta^{18}\text{O}$ and sea-level records reflect greatly extended marine-based ice caps is rejected. However, it is noted that the exact magnitude of temperature changes inferred from the isotopic

residuals is affected by (1) the glacio-eustatic accuracy of the sea levels; (2) the assumed ^{18}O -deficiency of the glacial ice and its variation with time.

Glacio-eustatism appears to be confirmed for the earlier high sea-level stands of the past 140 kyr (ref. 3) but the exact relationship between glacio-eustasy, global isostasy and sea-level changes during the late ice age stages, have yet to be resolved²⁵. However, the excellent agreement between the late high sea-level stands in New Guinea with estimates from areas with different tectonic histories^{12,13} argues against any significant non-glacio-eustatic component in the sea-level data. There are the uncertainties involved in the assumed magnitude and trend of ^{18}O depletion in the glacial ice that require further attention. The most prominent inferences as to the range of $\delta^{18}\text{O}$ ice and its effect on the isotopic temperature estimates are summarized in Table 2. The salient features of the tabulated results are as follows. First, an uncertainty of 2% in the assumed

Table 1 Amplitudes of late Quaternary oxygen isotope variations for the raised coral reefs in New Guinea and their palaeoclimatic interpretation

Reef phase†	Age‡ (kyr)	Sea level‡ (m)	No. of specimens§	$\delta^{18}\text{O}_{\text{PDB}} $ (‰)	$\delta^{18}\text{O}_{\text{sea level}} $ (m)	$\delta^{18}\text{O}_{\text{residual}} $ (‰)	$\Delta T(\delta)^*$ (°C)
Modern	0	0	10 (30)	-1.64 ± 0.05	—	—	—
Reef I crest	$*7 \pm 0.1$	-4 ± 1	6 (13)	-1.00 ± 0.07	-65	0.61	-2.7
Reef II crest	$*28.5 \pm 0.6$	-42 ± 1	1 (4)	-0.56 ± 0.17	-108	0.66	-2.9
Reef IIIb regn	37	-50	2 (6)	-0.31 ± 0.12	-132	0.82	-3.6
Reef IIIb crest	40	-39 ± 6	2 (6)	-0.80 ± 0.12	-84	0.45	-2.0
Reef IIIb transgn	$*42 \pm 3$	-47	2 (6)	-0.02 ± 0.12	-159	1.12	-4.9
Reef IIIa regn	43	-40	1 (3)	0.11 ± 0.17	-172	1.32	-5.7
Reef IIIa crest	45	-30 ± 6	1 (3)	-0.77 ± 0.17	-87	0.57	-2.5
Reef IIIa transgn	$*51 \pm 3$	-50	1 (3)	0.00 ± 0.17	-161	1.11	-4.8
Reef IV crest	$*60 \pm 4$	-28 ± 1	6 (18)	-0.67 ± 0.07	-97	0.69	-3.0
Reef V regn	81	-27	1 (1)	-1.05 ± 0.17	-71	0.44	-1.9
Reef V crest	$*85 \pm 4$	-13 ± 6	7 (15)	-1.34 ± 0.06	-32	NS	0
Reef V transgn	90	-38	1 (1)	-0.79 ± 0.17	-101	0.63	-2.8
Reef VI crest	$*107 \pm 6$	-15 ± 6	2 (8)	-1.44 ± 0.12	-22	NS	0
Reef VI transgn	112	-40	3 (6)	-0.91 ± 0.10	-87	0.47	-2.1
Reef VIIb regn	117	-10	7 (16)	-1.47 ± 0.06	-21	NS	0
Reef VIIb crest	$*120 \pm 3$	8 ± 3	7 (20)	-1.13 ± 0.06	-62	0.70	-3.0
Reef VIIa crest	$*133 \pm 4$	5 ± 5	8 (17)	-1.67 ± 0.06	4	NS	0
Reef VIIa transgn	143	-40	1 (3)	-1.24 ± 0.17	-41	NS	0
Reef VIIa transgn	148	-60	1 (4)	-0.80 ± 0.17	-84	NS	0

† Reefs are as in Fig. 1a: crest, culmination of a reef-building episode; transgn, transgressive shallow water facies; regn, regressive facies.

‡ Age estimates and sea levels according to refs 3, 14. Radiometric determinations are marked with an asterisk. Other dates are inferred from the stratigraphy¹⁴. Low sea-level uncertainties are estimated not to exceed ± 10 m.

§ *Tridacna gigas* individuals analysed per reef facies. Figures in parentheses indicate the number of isotope determinations, including chemistry.

|| Yearly average isotope compositions. Uncertainties are best estimates of the pooled standard error ($1\sigma_x$), allowing for the instrumental precision and for the number of giant clams analysed per reef³⁶.

¶ $\delta^{18}\text{O}_{\text{sea level}} = 3,800 [\Delta\delta^{18}\text{O}_{\text{reef}}/(\delta^{18}\text{O}_{\text{ice}} - \Delta\delta^{18}\text{O}_{\text{reef}})]$, where $\Delta\delta^{18}\text{O}_{\text{reef}} = \delta^{18}\text{O}_{\text{ancient}} - \delta^{18}\text{O}_{\text{modern}}$; $\delta^{18}\text{O}_{\text{residual}} = (\text{estimated sea level} - \delta^{18}\text{O}_{\text{sea level}}) \times 0.01$. NS, not statistically significant.

* Isotope temperature difference from present values (28.2 °C), $\Delta T(\delta) = \delta^{18}\text{O}_{\text{residual}}/0.23$.

Table 2 Effects of the $^{18}\text{O}/^{16}\text{O}$ ratio of glacial ice on the isotopic temperature estimates from the coral reefs in New Guinea during isotope stages 5 and 3

Method	$\delta^{18}\text{O}_{\text{SMOW}}$ of glacial ice (‰)		Temperature change (°C)*			
	Pre-70 kyr	Post-70 kyr	Early high† sea-level stands	Early low† sea-level stands	Late high‡ sea-level stands	Late low‡ sea-level stands
$^{18}\text{O}/^{16}\text{O}$ in ice cores of glacial ice relicts (Greenland and Antarctica) ²¹	-31	-37	0.0 (2)	-2.3 ± 0.5 (3)	-2.6 ± 0.5 (4)	-4.8 ± 0.9 (4)
Inference from D/H ratio of glacial-age trees in north-central North America ²⁷	-31	-22	0.0 (2)	-2.3 ± 0.5 (3)	-5.3 ± 0.7 (4)	-9.1 ± 1.3 (4)
$^{18}\text{O}/^{16}\text{O}$ residuals of planktonic foraminifera combined with ice caps reconstruction ²⁶	-31	-15	0.0 (2)	-2.3 ± 0.5 (3)	-8.3 ± 1 (4)	-13.8 ± 1.8 (4)

Values in parentheses indicate the number of pooled coral-reef units on which the temperature estimates are based (see Table 1). Interglacials are irrelevant to the discussion and are therefore omitted.

* Isotopic temperature estimates and the 1σ uncertainties using the equations set in Table 1.

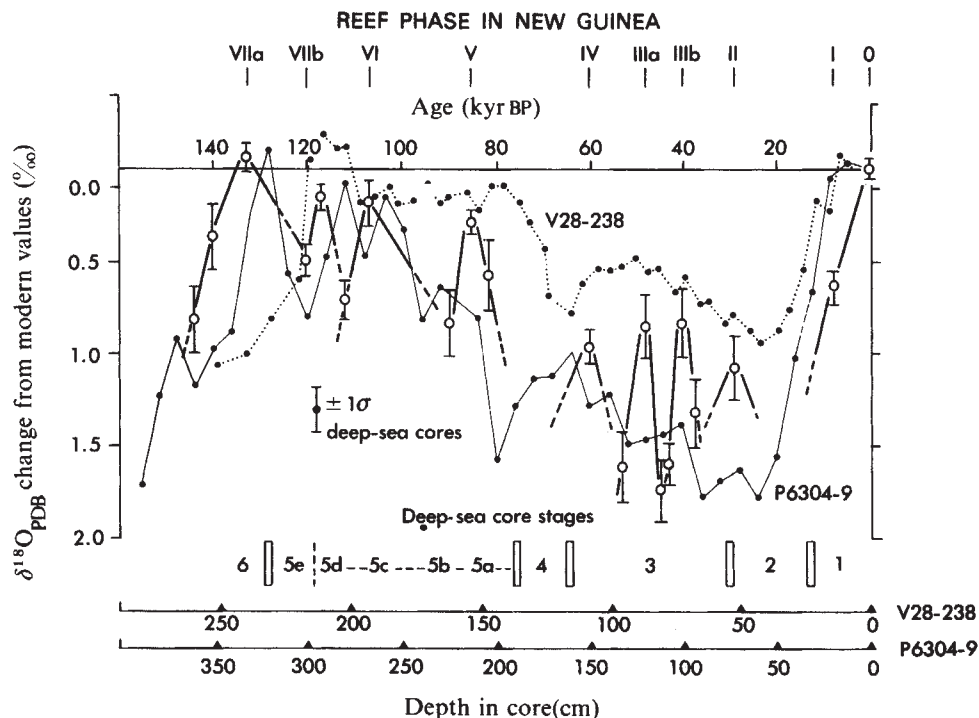
† Coral reefs VI and V (112 to 81 kyr).

‡ Coral reefs IV, III and II (60 to 28.5 kyr).

value of $\delta^{18}\text{O}$ ice propagates an error of 0.2 °C in the isotopic temperature estimates. Second, irrespective of the inferred range and trend of $\delta^{18}\text{O}$ ice, the temperature changes during isotope stage 3 differ significantly from the ones of isotope stage 5. Finally if $\delta^{18}\text{O}$ ice followed a trend of ^{18}O enrichment, as argued by Emiliani²⁶ and Yapp and Epstein²⁷, then the temperature estimates will differ from modern values (28.2 °C) by

as much as 5–8 °C during the late high sea-level stands and 9 to 14 °C during late, low sea-level stands. The inferred magnitude of cooling, however, is quite unlikely because it is substantially below the well established thermal stress threshold of coral-reef growth (~ 18 °C, ref. 28). It may be concluded therefore that (1) ^{18}O -deficiencies in the glacial ice, comparable with those observed in the ice cores of Greenland and Antarctic ice

Fig. 2 A comparison between $^{18}\text{O}/^{16}\text{O}$ data from *Tridacna gigas* of coral reefs in New Guinea and tropical deep-sea cores P6304-9 (Caribbean)¹⁷ and V28-238 (western equatorial Pacific)¹⁸ based on the planktonic foraminiferan *Globigerinoides sacculifera*. The three sequences are plotted to the same scale of isotopic change from present levels with δ -values adjusted to yield best agreement for the modern samples.



caps, provide conservative estimates for the total excess ice²¹; (2) $\delta^{18}\text{O}$ residuals in Table 1 and consequently the isotopic temperatures are maximum estimates for the temperature fall around New Guinea.

The results discussed here relate to the question of tropical ocean responses to high-latitude glaciations. Of special interest in this respect are the detailed history of the last interglacial event and the following periodic high sea-level stands for which the raised coral reefs contain a particularly good record. The interval which encompasses the last interglacial phase (133–120 kyr) includes a slow warming followed by a rapid cooling during the sea level rise at VIIb which has been attributed to an Antarctic surge¹⁰. There are no indications in the complete $\delta^{18}\text{O}$ -sea-level records in Fig. 1 for either a subsequent surge at 95 kyr (isotope stage 5), as proposed by Hollin²⁹, or multiple major surges during the following isotope stages^{30,31}. To the contrary, the evidence presented here supports an earlier contention¹⁰ that the cooling event, centred at the onset of the last ice age at 120 kyr, carries unique characteristics and its manifestation in the tropics waned while the northern glaciation was initiated. Thus the early warm interstadials generated by brief retreats of the incipient ice caps at 107 and 85 kyr seem to indicate that subsequent chilling of the climate was confined to the high latitudes. In contrast, later oscillations in the greatly expanded ice caps during isotope stage 3 must have affected the global climate as they are correlative to the cold interstadials at 60, 45 to 40 and 28 kyr. On this basis the evidence suggests that a considerable lag of ~40,000 yr occurred between the initiation of the continental ice and sea-surface temperature variations in the tropical Pacific. As the last glaciation maximum (~18 kyr) has not been sampled, it cannot be established from the coral-reef record whether the maximum temperature fall during isotope stage 3 exceeded that of isotope stage 2 although Geitzenauer *et al.*²³ suggest that it does. If so, the nonlinearity in the relative contributions of temperature and ice volume bespeaks a complexity in the tropical marine oxygen isotope records greater than has been previously recognized^{18,32}.

In view of the climatic significance attached to the thermal history of the tropical Pacific^{33,34} the techniques developed in this study should be applied to other sites where late Quaternary coral reefs have been reported^{12,35}, therefore broadening the regional and global extent of the coral-reef palaeoclimatic record.

I thank J. Chappell and W. Compston for introducing me to the geology of the Huon reefs and mass spectrometry respectively; people of the villages Sialum, Gitua, Kalasa and Paukwanga on the Huon Peninsula for logistic help and hospitality. This study was supported by an ANU Scholarship and a Queen Fellowship in Australia.

Received 12 April; accepted 16 June 1983.

1. Darwin, C. in *The Structure and Distribution of Coral Reefs* 1842 (University California Press, Berkeley and Los Angeles, republished 1962).
2. Mesolella, K. J., Matthews, R. K., Broecker, W. S. & Thurber, D. L. *J. Geol.* **77**, 250–274 (1969).
3. Bloom, A. L., Broecker, W. S., Chappell, J., Matthews, R. K. & Mesolella, K. J. *Quat. Res.* **4**, 185–205 (1974).
4. Bloom, A. L. in *Earth Rheology, Isostasy and Eustasy* (ed. Morner, N. A.) 505–516 (Wiley, New York, 1980).
5. Fairbanks, R. G. & Matthews, R. K. *Quat. Res.* **10**, 181–196 (1978).
6. Shackleton, N. J. & Matthews, R. K. *Nature* **268**, 618–619 (1977).
7. Chappell, J. *Bull. geol. Soc. Am.* **85**, 553–570 (1974).
8. Veeh, H. H. & Chappell, J. *Science* **167**, 862–865 (1970).
9. Chappell, J. & Polach, H. *Bull. geol. Soc. Am.* **87**, 235–240 (1976).
10. Aharon, P., Chappell, J. & Compston, W. *Nature* **283**, 649–651 (1980).
11. Stuiver, M., Heusser, C. J. & Yang, I. C. *Science* **200**, 16–21 (1978).
12. Chappell, J. & Veeh, H. H. *Bull. geol. Soc. Am.* **89**, 356–368 (1978).
13. Konishi, K., Omura, A. & Nakamichi, O. in *Proc. Sec. int. Coral Reef Symp. Brisbane 2*, 595–613 (1974).
14. Aharon, P. thesis, Australian National University, Canberra (1980).
15. Craig, H. *Geochim. cosmochim. Acta* **12**, 133–149 (1957).
16. Epstein, S., Buchsbaum, R., Lowenstam, H. & Urey, H. C. *Bull. geol. Soc. Am.* **64**, 1315–1326 (1953).
17. Emiliani, C. *J. Geol.* **74**, 109–126 (1966).
18. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39–55 (1973).
19. Robin, G. De Qu. *Phil. Trans. R. Soc. B* **280**, 143–168 (1977).
20. Johnsen, S. J., Dansgaard, W., Clausen, H. B. & Langway, C. C. *Nature* **235**, 429–434 (1972).
21. Dansgaard, W. & Tauber, H. *Science* **166**, 499–502 (1969).
22. Broecker, W. S. *Science* **188**, 1116–1118 (1975).
23. Geitzenauer, K. R., Roche, M. B. & McIntyre, A. *Geol. Soc. Am. Mem.* **145**, 423–448 (1976).
24. Williams, D. F., Moore, W. S. & Fillon, R. H. *Earth planet. Sci. Lett.* **56**, 157–166 (1981).
25. Nakiboglu, S. M., Lambeck, K. & Aharon, P. *Tectonophysics* **91**, 335–358 (1983).
26. Emiliani, C. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 183–197 (Yale University Press, New Haven, 1971).
27. Yapp, C. J. & Epstein, S. *Earth planet. Sci. Lett.* **34**, 333–350 (1977).
28. Roberts, H. H., Rouse, L. J., Walker, N. D. & Hudson, J. H. *J. Sedim. Petrol.* **52**, 145–155 (1982).
29. Hollin, J. T. *Nature* **283**, 629–633 (1980).
30. Hollin, J. T. *Am. Quat. Ass. 6th Biennial Meet. Abstr. Prog.* 102 (1980).
31. Flohn, H. in *Antarctic Glacial History and World Palaeoenvironments* (ed. Van Zinderen Bakker, E. M.) 3–13 (Balkema, Rotterdam, 1978).
32. Emiliani, C. *J. Geol.* **63**, 538–578 (1955).
33. Manabe, S. & Hahn, D. G. *J. geophys. Res.* **82**, 3889–3911 (1977).
34. Newell, R. E., Navato, A. R. & Hsiung, J. *Pageophysics* **116**, 351–371 (1978).
35. Mitchell, A. H. G. *J. Geol.* **76**, 56–67 (1968).
36. Aharon, P., Chappell, J. & Compston, W. in *Carbon Dioxide Effects: Research and Assessment Program* (ed. Jacoby, G. C.) 94–101 (Lamont-Doherty Geological Observatory, 1980).

Dominance and reproductive success among slave-making worker ants

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Dominance hierarchies are of great theoretical interest because they result from a compromise between selfish and social behaviour. In a wide variety of vertebrates dominance promotes individual fitness¹, but often to the detriment of the group as a whole. For example, in gelada baboons, females at the top of the hierarchy produce more offspring because their ovulations are less frequently inhibited and they have fewer abortions than their more harassed subordinates². This type of selfish behaviour is perhaps least expected within ant colonies because their workers are exceptionally closely related³ and worker inclusive fitness is largely determined by the productivity of the colony as a whole⁴. For this reason our discovery of primate-like linear dominance hierarchies within colonies of slave-making ants uniquely demonstrates the importance of individual selection in social evolution. High ranking slave-maker workers receive more food from their slaves, have greater ovarian development and do less foraging than their subordinate sisters. This is also the first demonstration that ant queens can recognize the rank of their workers. Experiments show that the queen solicits food preferentially from the most dominant of her workers, possibly to limit their production of eggs. We explain these phenomena in terms of competition for the production of sons.

The ant *Harpagoxenus americanus* is an obligate slave-maker and uses workers of certain *Leptothorax* species as its labour force⁵. Colonies of *H. americanus* have one queen usually with no more than 10 slave-maker workers and up to 200 slaves^{6,7}. Typically they inhabit hollow acorns and they can be rehoused in nests made of microscope slides. This allows an entire colony to be viewed through a dissection microscope so that all social interactions within the nest can be observed. The study colonies were collected in the spring of 1982 in Massachusetts and they were cultured in standard conditions⁵. All the slave-making workers in each colony were individually marked with fast-drying paint⁸. Observations were made during May, June and July of 1982.

Antagonistic interactions between slave-maker workers take several forms from avoidance to ritualized fighting. For example, one worker may abruptly run from another following

antennal contact. On other occasions one ant will drum its antennae over the head and thorax of another until the submissive workers crouches still, when the aggressor may actually climb on top of its subordinate. Such encounters often result in low-ranking workers being pinned in place for several minutes at a time. Similar interactions also occur between the queen and her workers. Tables 1 and 2 show examples of dominance hierarchies in two colonies of *H. americanus*. These tables present pooled data from three distinct types of interaction: (1) one ant fleeing from another; (2) encounters in which one ant was clearly an aggressor; and (3) feeding of one ant by another. The hierarchies are almost perfectly linear: only 1% of all interactions were reversals.

Dominance behaviour appears to be intimately associated with competition for food within the slave-maker colony. *H. americanus* workers can only obtain food from their slaves; they do not feed each other and cannot forage for food⁷. The proximate advantage of high rank is that dominant workers are fed more often by the slaves (Table 2). The explanation for this is that confrontations often began when a dominant worker interrupted liquid food exchange between one of its subordinates and a slave and such encounters often ended when the dominant worker was fed by a slave. A subordinate slave-maker was never seen to be fed in the presence of one of its more high-ranking sisters. As adult slave-maker workers do not grow we expected dominant workers to invest their extra energy supplies in the production of eggs. This is indeed the case. Standard dissections⁹ of all the slave-maker workers in colony 1 revealed a significant correlation between rank and ovarian development (Spearman's $r_s = 0.94$, $P < 0.05$; Table 1). The presence of corpora lutea at the base of the ovarioles of these workers showed that they were laying the eggs they were producing⁶ and it is unlikely that these eggs were being used as food for the colony, as is the case in some other ants¹, because these workers were themselves competing for food. Furthermore, the haploid eggs that *H. americanus* workers produce by parthenogenesis develop into males in the same way as do the unfertilized eggs laid by the queen^{6,7}. Hence we suggest that the ultimate advantage of dominance is that high-ranking workers are able to produce more sons than subordinate workers.

This is an important phenomenon because workers that are mothers will be in conflict with the queen as she would prefer all the resources of her colony to be invested in her own progeny. The workers that will be in greatest conflict with the queen will be those highest in the dominance hierarchy. Most intriguingly, it is predominantly these high-ranking slave-maker workers from whom the queen solicits and receives food (Table 2). Moreover, these are the only occasions when slave-makers exchange food. Bouts of feeding of the queen by her slave-maker workers lasted for a much longer time than bouts of food exchange from the slaves. That the queen demands that she be fed by her dominant workers yet rarely even solicits food from her low-ranking workers suggests that she may be acting to arrest ovarian development in those of her daughters

Table 1 Dominance hierarchy tabulated from 10 h of observation

Dominant ants	Subordinate ants							Total times dominating	Ovarian development	
	Queen	P	GA	T	GR	B	W		Eggs	Active ovarioles
Queen	—	7	9	1	3	3	4	27	—	—
P		—	41	54	28	32	49	204	5	5
GA		1	—	22	24	26	24	97	4	4
T				—	18	27	38	83	2	3
GR				2	—	22	16	40	1	3
B						—	7	7	0	0
W						1	—	1	1	1
Total times dominating	0	8	50	79	73	111	138			

Each entry is the number of interactions between the indicated pair of ants, colony 1. The positive correlation between the number of times a worker dominates and its rank is significant (Spearman's $r_s = 1$, $P < 0.05$). The negative correlation between the number of times a worker is dominated and its rank is significant (Spearman's $r_s = -0.943$, $P < 0.05$).

Table 2 Dominance hierarchies before the removal, in the absence, and after the return, of the dominant worker (colony 2)

Initial observation of 27 h						No of times fed by slaves
Dominant ants	Subordinate ants					
	Queen	M	L	F	O	
Queen	—	41	25	13	7	90
M		—	75	40	21	98
L			—	29	18	50
F				—	2	55
O					—	54
Queen fed by slave-maker workers		8	0	0	1	

After removal of M, 10-h observation						
	Queen		L	F	O	
Queen	—		20	8	5	28
L			—	24	24	18
F				—	10	13
O					—	21
Queen fed by slave-maker workers			1	1	1	

After return of M, 10-h observation						
	Queen	M	L	F	O	
Queen	—	8	3	3	0	31
M		—	48	39	9	49
L			—	29	4	15
F			3	—	10	14
O				3	—	9
Queen fed by slave-maker workers		4	1	0	0	

that most threaten her fitness. An experiment in which the most dominant worker was removed (see Table 2) also indicates that the queen recognizes that status of her workers. This experiment had two major results: there was a significant increase in the frequency of antagonistic interactions within the colony (paired t -test: $t = 3.98$, $P < 0.02$) and the queen took food for the first time from those workers that moved to the 'alpha' and 'beta' positions in the worker hierarchy. When the original alpha worker was returned to the colony, she came into conflict with all her sisters at a significantly higher rate than she did before her removal ($\chi^2 = 4.15$, $P < 0.05$) and she directed her aggression predominantly at the worker that had usurped her alpha position. The returned worker regained her old supremacy and was again required to feed the queen.

The other major activity of slave-maker workers is concerned with the procurement of slaves. Slave-maker workers never forage for food; apparently they only ever leave the nest either to scout for *Leptothorax* colonies or to participate in slave raids against such colonies⁷. Of these activities scouting is perhaps the most dangerous because it is undertaken by individual slave-maker workers who, unaided by their sisters, will be attacked by any *Leptothorax* workers they discover. We observed 39 separate bouts of scouting and without exception they were conducted by the most subordinate of the slave-maker workers. This uneven division of labour and risk-taking among the slave-maker workers can be interpreted in two ways. First, a subordinate worker, who is least likely to become a mother when bullied by her sisters, has least to lose by altruistically taking the risks associated with foraging. Second, the scouting worker may be able to produce sons elsewhere or at least increase her inclusive fitness by having her sisters produce more sons in a new nest.

On certain occasions after a slave raid some of the slave-maker workers do not return to their home nest^{6,7}. These *H.*

americanus workers remain in the raided *Leptothorax* nest with the brood they have captured. This brood will develop into a new slave labour force whereupon the slave-maker workers can start to produce sons and indeed such secondary nests are known to produce exceptionally male-biased broods^{6,7}. Thus, by instigating a slave-making raid a subordinate worker may still be able to become a mother. In addition, a subordinate worker may be able to increase her inclusive fitness if by her actions her sisters produce more sons. This is because workers who have the same father will be more closely related to their nephews than to their brothers as males are haploid and females are diploid. This means that any worker should prefer to have her own sons raised by the colony but if this is not possible she should prefer any worker-produced male to a son of the queen. For this reason a slave raid that produces a new nest may function more as a strategic mutiny than as an altruistic act that serves the queen.

We thank E. O. Wilson, Bert Holldobler, T. M. Alloway and S. H. Bartz for their encouragement and advice. Mark Elgar, Bert Holldobler and Stuart Reynolds made valuable criticisms of an earlier draft of this paper. This research was supported by a fellowship from the Royal Commission for the Exhibition of 1851 to N.R.F. and NSF grant DEB 77-27515 to E. O. Wilson.

Received 25 April; accepted 21 June 1983.

1. Wilson, E. O. *Sociobiology, The New Synthesis* (Belknap, Cambridge, 1975).
2. Dunbar, R. I. M. & Dunbar, E. P. *Nature* **266**, 351–352 (1977).
3. Hamilton, W. D. *J. theor. Biol.* **7**, 1–52 (1964).
4. Oster, G. F. & Wilson, E. O. *Caste and Ecology in Social Insects* (Princeton University Press, 1978).
5. Alloway, T. M. *Am. Nat.* **115**, 247–261 (1980).
6. Buschinger, A. & Alloway, T. M. *Psyche, Camb.* **83**, 233–242 (1977).
7. Wesson, L. G. Jr *Trans. Am. ent. Soc.* **65**, 97–122 (1939).
8. Porter, S. D. & Jorgensen, C. D. *Ecol. Ent.* **5**, 263–269 (1980).
9. Cole, B. J. *Science* **212**, 83–84 (1981).

Naloxone augments electrophysiological signs of selective attention in man

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Previous research on the behavioural functions of endogenous opioid systems in rodents suggested a possible opioid role in the regulation of attention^{1,2}. This proposal was consistent with reports that opiate administration in man impairs the ability to concentrate³ while opiate antagonists augment behavioural⁴ and electrophysiological⁵ indices of arousal and attention. We examined the effects of the opiate antagonist naloxone on electrophysiological measures of attention in normal human subjects, using a paradigm which dissociates selective information processing from concurrent processes of general arousal or alertness that may be present⁶. We now report electrophysiological evidence that naloxone improves the selectivity of auditory attention in the presence of competing sources of stimuli. These findings indicate a role for the endogenous opioid systems in the regulation of selective attention in man.

Electrophysiological measures of selective attention were obtained in a selective listening paradigm developed by Hillyard and associates^{6–8}. Auditory event-related potentials (AERPs) were recorded from subjects who listened selectively to

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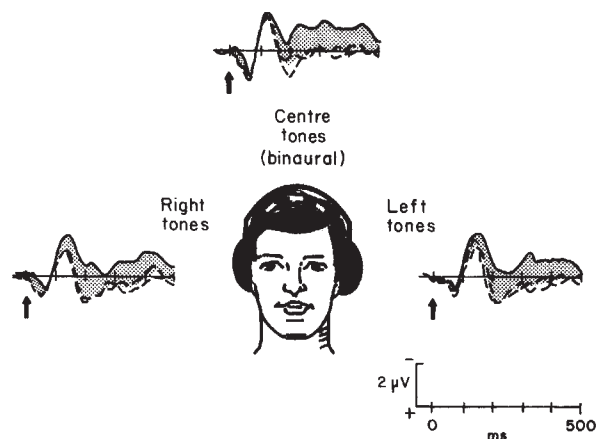


Fig. 1 Diagram representing the perceived locations of the stimulus channels and showing the grand average AERPs (for 10 subjects) elicited by the right, centre and left standard tones following placebo administration. Auditory event-related potentials (AERPs) to attended channel tones (solid lines) and inattended channel tones (broken lines) were recorded at the frontal midline electrode site (Fz). The attention effect is illustrated by stippling.

sequences of discrete tones delivered in random order through earphones to one of three perceived spatial locations; left ear, right ear and centre of the head (binaural). All tones were 1,000 Hz tone pips randomly delivered at intervals of 200–400 ms at an intensity of 40 dB sound level. Tones at each location were attended in turn, and subjects were instructed to detect occasional 'target' tones of a slightly longer duration (40 ms) than the 'standard tones' (25 ms) in the attended 'channel'.

The physiological measure of selective attention was the difference between the AERP to attended-channel tones and the AERP to the same sequence of tones when they were not attended. In these conditions, the AERPs to attended tones typically show an increased negativity over the latency range 100–500 ms post-stimulus, which has been interpreted as an index of the selective processing that is accorded attended-channel stimuli following an early (stimulus set) selection^{9,10}. This 'attention effect' consists of two phases, an early negative shift superimposed on the evoked N1 wave at ~100 ms, and a later, prolonged negativity with a more frontal scalp distribution¹⁰. The amplitude of the attention effect roughly correlates with the subject's accuracy in discriminating target stimuli in the channel^{7,8}. If naloxone enhances this type of selective attention in man, we would expect it to augment the AERP attention effect and to improve behavioural performance on the three-channel listening task.

Young men (20–28 yr old) were injected with 2 mg naloxone (that is, approximately 0.03 mg per kg) intravenous over 2 min on one experimental day and with a placebo on another day according to a double-blind, cross-over design. They were tested in the selective attention paradigm at 10 min post-injection. During each session the subject was presented with five task conditions in counterbalanced order: (1) selective attention to the left channel; (2) selective attention to the right channel; (3) selective attention to the centre channel; (4) divided attention (detect targets in all three channels at once); and (5) undistracted attention to the centre channel (lateral channels turned off). Total testing time was 50–70 min and thus occurred within the effective range of the actions of naloxone. Electroencephalographic activity was recorded from frontal, central and parietal midline sites (Fz, Cz, Pz), and from two lateral frontal sites (F3 and F4), using Ag–AgCl scalp electrodes. Recordings of electroocular activity from the lower orbital ridge showed the AERP data to be free from ocular artefact.

Findings from placebo sessions replicated earlier reports, that is, the AERPs to attended channel tones showed an enlarged

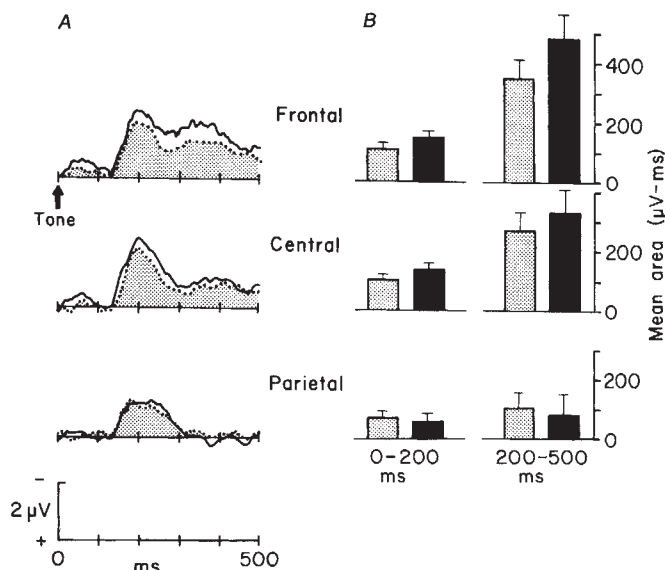


Fig. 2 The effects of 2 mg naloxone on the AERP attention effect averaged over left and right channel tones at midline electrode sites (Fz, Cz, Pz). **A**, The difference between the attend and inattend waveforms (the attention effect) following placebo (dotted lines) or naloxone (solid lines) administration. **B**, The mean area of the attention effect ($\pm$ s.e.) for the early (0–200 ms) and late (200–500 ms) epochs after stimulus onset. Naloxone (solid bars) produced a significant augmentation of the late attention effect at frontal electrode sites.

negative component beginning at ~100 ms in relation to the AERPs to inattended tones (Fig. 1). The amplitude of this attention effect was measured for the standard tones by averaging the waveforms for each channel of stimuli over the two non-attend conditions and subtracting this average from the attended waveform (represented by stippled area in Fig. 1). The area of this difference wave (in μ V·ms) was measured from 0 to 200 ms (early attention effect) and from 200 to 500 ms (late attention effect) after stimulus onset. A highly significant attention effect was demonstrated over all three channels for the early and late epochs (attention main effect: early, $F(1,9) = 21.56$, $P < 0.002$; late, $F(1,9) = 22.65$, $P < 0.001$, three-way analysis of variance with repeated measures (ANOVA-R) where factors were attention, channels and electrodes. The late attention effect had a more frontal distribution on the scalp (Fig. 2). Also consistent with previous reports^{7,8}, the early attention effect to the centre tones at anterior electrode sites tended to be smaller than the attention effects to the lateral channels. This finding has been interpreted as resulting from the poorer discriminability of the centre channel^{7,8}.

Naloxone significantly augmented the attention effect at frontal electrode sites for the 200–500 ms epoch (drug $\times$ electrode interaction: $F(4,36) = 4.57$, $P < 0.005$, three-way ANOVA-R with factors of drug, channels and electrodes). The naloxone-related increase of the early attention effect was not significant (drug $\times$ electrode interaction: $F(4,36) = 1.46$, $P < 0.30$). Further analysis revealed that the naloxone-induced augmentation of the late attention effect was significant over the combined left and right stimulus channels (Fig. 2; drug $\times$ electrode interaction: $F(4,36) = 4.37$, $P < 0.006$, two-way ANOVA-R), but not for the centre tones (drug $\times$ electrode interaction: $F(4,36) = 1.14$, $P < 0.40$, two-way ANOVA-R). Nine of the 10 subjects tested displayed a larger combined left and right attention effect at the right frontal (F4) electrode site following naloxone administration than for the placebo. There was no significant difference between left and right frontal electrodes. The augmentation of the attention effect resulted primarily from naloxone producing a large positive shift of the inattend waves (drug $\times$ electrode interaction: $F(4,36) = 5.68$, $P < 0.002$, two-way ANOVA-R),

and a slight negative shift of the attend waves (drug: $F(1,19) = 3.47$, $P < 0.10$, two-way ANOVA-R).

The subjects' behavioural performance in discriminating the target tones within the attended channel was nearly perfect with mean detection sensitivity (d') scores above 3.0 in all conditions. Although these ceiling effects prevented a sensitive evaluation of the effects of naloxone on target detection, naloxone did produce a small but significant improvement for the attend left condition (d' scores of 4.91 versus 3.71, $t = 2.72$, $P < 0.05$). Further testing with more sensitive measures of attentional selectivity is needed to determine more reliably the effects of naloxone on performance.

The effects of naloxone seemed to be specific to the selective aspects of information processing. An estimate of overall 'attentional capacity', as reflected in the summed negativity over all three stimulus channels during the divided attention condition^{11,12}, showed no effect of naloxone. There was also no drug effect on the AERPs or behavioural performance in the undistracted attention condition. Measures of blood pressure, pulse rate, auditory thresholds and reaction times for target detections were similarly uninfluenced by naloxone. Evaluations of the effects of naloxone on mood and arousal using standard psychiatric self-report scales showed no significant drug effects. These findings make it unlikely that the increase in attentional selectivity produced by administration of naloxone was mediated by an overall change in arousal level, which would be expected to produce more widespread changes in these behavioural and autonomic indices.

These findings suggest that naloxone can affect a person's ability to allocate attention to relevant sources without altering total processing capacity or global arousal levels. Whether this increase in selective attention is due to a sustained narrowing of the focus of attention or to a decrease in attentional lability (distractibility) cannot be determined from these results. However, as the augmentation of the attention effect resulted more from a large positive shift in the inattent waves than from a negative shift of the attend waveform, it is possible that naloxone improves selective attention primarily by suppressing attention to distracting stimuli. Furthermore, as naloxone did not alter the attention effect to the less discernible centre tones, it is possible that naloxone facilitates the suppression of distracting stimuli only when those stimuli can be readily discriminated from the relevant tones.

The pattern of naloxone binding in the primate cortex has been interpreted as suggesting an opioid role in selective attention^{13,14}. Areas such as the frontal cortex which have been implicated in the regulation of selective attention¹⁵ are rich in naloxone binding sites and may contain intrinsic enkephalin-immunoreactive neurones¹⁶. Our previous research with rodents indicated that naloxone affected stimulus-directed behaviour through actions at opiate receptors². Similarly, the naloxone-induced augmentation of the attention effect may result from an antagonism of endogenous opioid activity, suggesting that the endogenous opioids may participate in the regulation of selective attention in humans. These observations raise the possibility that opiate antagonists may prove effective in the treatment of attention deficit disorders.

We thank Endo Laboratories for naloxone, Don Lawson for technical expertise and Regina Ciabrone, Marta Kutas, Greg Chesney, Jonathan Hansen and Steve Van Voorhis for their assistance. This work was supported in part by USPHS grants DA-01994-05, MH-30914-06, MH-25594 and AA-03504. D.S.S. is the recipient of USPHS Career Scientist Award MH-70183-10.

Received 14 February; accepted 15 June 1983.

1. Arnsten, A. T. & Segal, D. S. *Life Sci.* **25**, 1035-1042 (1979).
2. Arnsten, A. F. T., Segal, D. S., Loughlin, S. E. & Roberts, D. C. S. *Brain Res.* **222**, 351-363 (1981).
3. Jaffe, J. H. & Martin, J. R. in *The Pharmacological Basis of Therapeutics* 6th edn (eds Gilman, A. G., Goodman, L. S. & Gilman, A.) 494 (Macmillan, New York, 1980).
4. Gritz, E. R., Shiffman, S. M., Jarvik, M. E., Schlesinger, J. & Charuvastra, V. C. *Clin. Pharmac. Ther.* **19**, 773-776 (1976).
5. Davis, G. C., Buchsbaum, M. S. & Bunney, W. E. in *Neural Peptides and Neuronal Communications* (eds Costa, E. & Trabucchi, M.) 473 (Raven, New York, 1980).

6. Hillyard, S. A., Hink, R. F., Schwent, V. L. & Picton, T. W. *Science* **182**, 177-180 (1973).
7. Schwent, V. L., Snyder, E. & Hillyard, S. A. *J. exp. Psychol.: Hum. Perception Performance* **2**, 313-325 (1976).
8. Parasuraman, R. *Psychophysiology* **15**, 460-465 (1978).
9. Naatanen, R. & Michie, M. T. *Biol. Psychol.* **8**, 81-136 (1979).
10. Hansen, J. & Hillyard, S. A. *Electroenceph. clin. Neurophysiol.* **49**, 277-290 (1980).
11. Hink, R. F., Van Voorhis, S., Hillyard, S. A. & Smith, T. S. *Neuropsychologia* **15**, 597-605 (1977).
12. Okita, T. *Biol. Psychol.* **9**, 271-284 (1979).
13. Lewis, M. E. *et al. Science* **211**, 1166-1169 (1981).
14. Wise, S. P. & Herkenham, M. *Science* **218**, 387-389 (1982).
15. Knight, R. T., Hillyard, S. A., Woods, D. L. & Neville, H. *Electroenceph. clin. Neurophysiol.* **50**, 112-124 (1980).
16. McGinty, J. F., van der Kooy, D., Koda, L. Y. & Bloom, F. E. *Anat. Rec.* **202**, 125a (1982).

Antibodies to paired helical filaments in Alzheimer's disease do not recognize normal brain proteins

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During ageing of the human brain, and particularly in senile dementia of the Alzheimer type (AD), many neurones progressively accumulate abnormal cytoplasmic fibres, called paired helical filaments (PHF)¹⁻³. Each such fibre consists of a pair of intermediate (10 nm) filaments twisted into a double helix with a periodicity of 160 nm. PHF accumulate in large perikaryal masses, called neurofibrillary tangles, and are also found in degenerating cortical neurites that form neurite plaques. The density of PHF-containing neurites and cell bodies correlates with the degree of dementia⁴⁻⁶ and the extent of loss of cholinergic neurotransmitter function⁷ in AD. Recently, we demonstrated that PHF from human cerebral cortex are large, rigid polymers with unusual molecular properties, including insolubility in SDS, urea and other denaturing solvents⁸ and apparent resistance to protease digestion⁹. These properties have so far prevented complete purification and analysis of the constituents of PHF. Based on their insolubility, we have developed a new method of preparing highly enriched PHF fractions and have raised an antiserum that is highly specific for PHF. We report here that this antiserum specifically labels PHF, free of any associated normal fibrous proteins and, unexpectedly, it reacts with neither neurofilaments nor any other normal cytoskeletal protein in brain sections or on immunoblotted gels. These anti-PHF antibodies have been used for the specific detection of Alzheimer-type PHF and in the search for cross-reacting antigens in various tissues, and are suitable for immunoaffinity purification of PHF. Our results indicate that PHF contain determinants that are not shared with normal neuronal fibrous proteins.

To purify PHF, 30-40 g of frozen AD cortex rich in neurofibrillary tangles were finely chopped, incubated at 25 °C for 2 h in 50 mM Tris pH 7.6, 0.1 M β -mercaptoethanol (β -ME), 2% SDS (SDS/ β -ME buffer), Dounce-homogenized and heated to 100 °C for 5 min. The homogenate was sieved once through nylon meshes (110 and 64 μ m) and centrifuged at 100,000g for 30 min. The pellets were suspended in the same buffer containing 1% SDS and spun at 200g for 10 min. The supernatant was spun at 100,000g for 30 min; the resultant pellets were resuspended in 1% SDS buffer and layered on gradients composed of 1.0, 1.2, 1.4 and 2.0 M sucrose layers in 50 mM Tris, 1% SDS. After centrifugation at 243,000g for 2 h at 25 °C, abundant neurofibrillary tangles were recovered at the 1.4-2.0 M interface, which was diluted and pelleted (100,000g, 60 min). Electron microscopy (EM) revealed that PHF were by far the major constituent of this final pellet and that their fine structure was unchanged from PHF *in situ*. No

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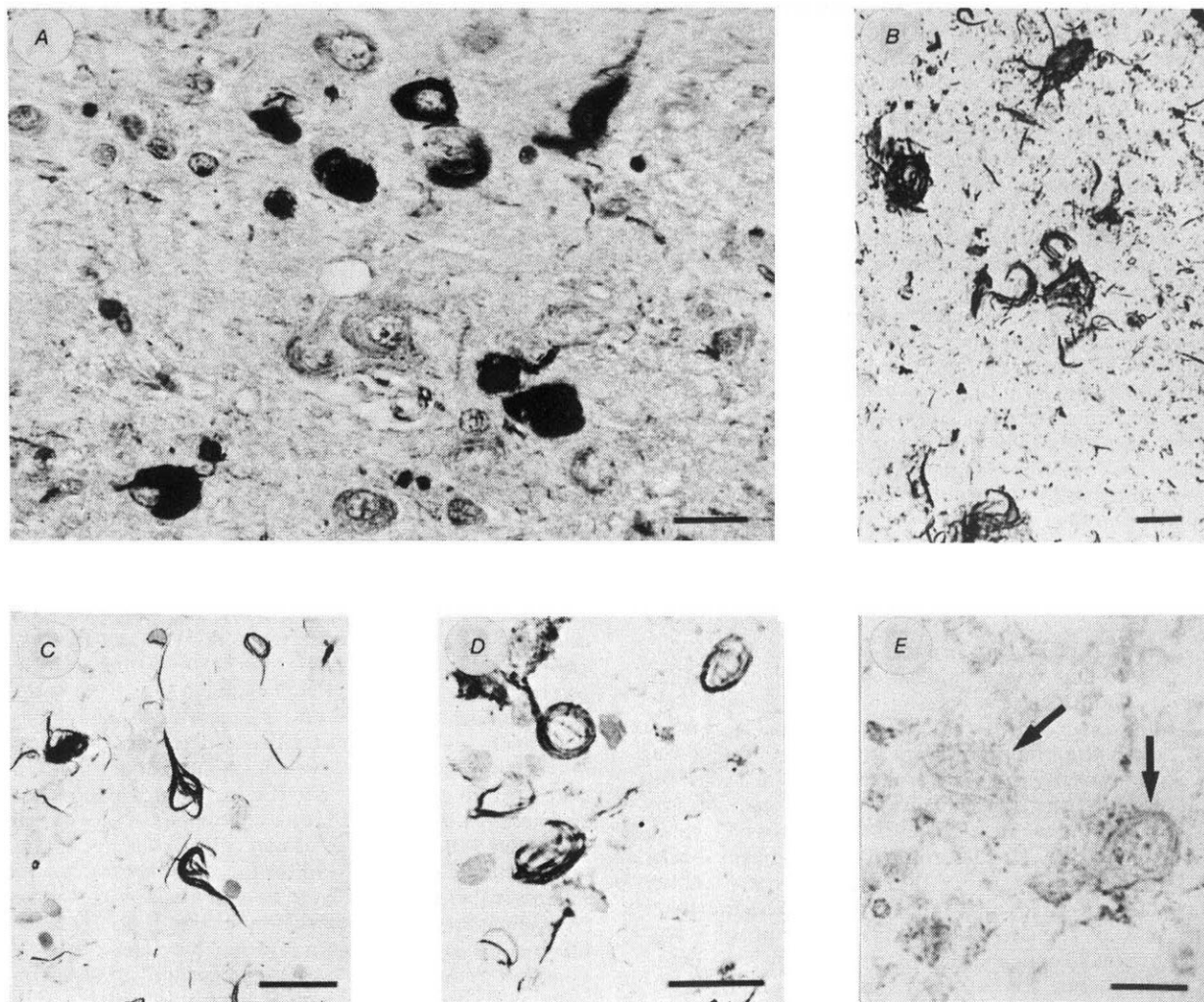


Fig. 1 Light micrographs of *in situ* and isolated neurofibrillary tangles (NFT) stained with anti-PHF antiserum. **A**, Paraffin section of formalin-fixed AD hippocampal cortex stained by the peroxidase-antiperoxidase (PAP) method and counterstained with haematoxylin. Several NFT are darkly stained at a 1:1,000 dilution of the primary antiserum. **B**, NFT isolated by extensive extraction and heating of AD cortex in SDS/ β -ME were pelleted and 4- μ l aliquots were placed on albumin-coated slides and stained at 1:1,000 (PAP method). **C**, NFT isolated by extraction of AD cortex in Tris-buffered saline (TS) and stained at 1:1,000 (PAP method). **D**, TS-isolated NFT stained with anti-PHF antiserum (1:500) absorbed with temporal cortex from 92-yr-old normal brain (case 10 in Table 1). **E**, Same as **D**, but absorbed with NFT-rich temporal cortex of AD brain (case 2 in Table 1). Arrows indicate unstained NFT. **D**, **E**, Indirect peroxidase method was used. Scale bars, 10 μ m. For details of absorption technique, see Table 1.

neurofilaments or other normal fibrous organelles were present. Lipofuscin granules were the principal contaminant. Protein yield of the final fraction was 50–80 μ g per g wet wt of tangle-rich starting cortex.

Two rabbits were injected subcutaneously with 200 μ g of the purified PHF pellet emulsified in complete Freund's adjuvant. The pellet had been sonicated for 40 s at 50 V and consisted of small (50–350 nm) PHF fragments. Four weeks later, the rabbits received a booster of 200 μ g PHF pellet in incomplete Freund's adjuvant. Sera taken from both rabbits 1 week after the second injections strongly immunolabelled many neurofibrillary tangles in formalin-fixed sections of AD cortex (Fig. 1A). The antiserum of one rabbit (used in all subsequent experiments) stained intraneuronal masses displaying the forms characteristic of AD tangles in hippocampal and neocortical neurones at up to 1:5,000 dilution. Comparison of immunolabelled sections with adjacent silver-stained sections indicated that a high percentage of silver-positive tangles reacted with the antiserum. The antiserum also labelled many abnormal neurites scattered diffusely throughout the neuropil. It stained some of the neurites in neuritic plaques; however, the antiserum detected fewer neuritic plaques than did silver

staining. In sections of normal human cerebrum and normal and AD cerebellum, no structures were specifically immunolabelled; in particular, neurofilament-rich axons around Purkinje cells and neuronal fibres in the cerebellar molecular layer and white matter were not stained. The neurofibrillary tangles found occasionally in normal aged human cortex were stained identically to AD tangles.

To determine whether our antiserum directly labelled PHF or associated normal fibrous proteins in the tangles, we reacted the antiserum with isolated tangles that had been extensively extracted in SDS/ β -ME. The SDS/ β -ME-insoluble tangles, which retained their characteristic fibrous form, were stained by the antiserum at up to 1:5,000 dilution (Fig. 1B). In contrast, an antiserum against gel-purified 200,000 (200K) molecular weight (MW) rat neurofilament protein, which had previously been shown to label AD tangles *in situ*¹⁰, no longer stained tangles after SDS extraction. As the only fibrous proteins remaining in such SDS/ β -ME-purified tangles were PHF (as judged by both EM and gel electrophoresis), the results indicate that the antibodies in our serum directly labelled PHF. Extensive absorption experiments with various AD and control (normal and diseased) brain tissues showed that the anti-PHF

Table 1 Absorption of tangle immunoreactivity by various brain homogenates

Case	Age (yr)	Neuropathological diagnosis	Brain area	Change in tangle staining	Tangle content of absorbant	Neuritic plaque core content of absorbant
1	49	Down's syndrome with AD	T	↓↓	4+	+
2	85		F	↓↓	3+	+
			T	↓↓	4+	+
3	72	AD	F	↓↓	4+	±
4	74	AD	F	↓↓	2+-3+	2+-3+
			T	↓↓	2+-3+	3+-4+
5	62	AD	F	↓~	±	±+
			T	—	0	+
6	96	AD	F	↓	±	2+
			T	↓↓	2+-3+	+
7	83	AD	F	↓	±+	+
			T	↓	2+	±
8*	75	AD	Cb	—	0	±
9	85	Normal	F	—	0	0
			T	—	0	0
10	92	Normal	F	—	0	+
			T	—	0	±
11	60	Normal	F	—	0	0
			T	—	+	0
			Cb	—	0	0
12	75	Leukodystrophy	F	—	0	0

T, inferior temporal cortex; F, orbitofrontal cortex; Cb, cerebellum. —, No change; ↓, moderate decrease; ↓↓, marked decrease. In each case, 12 µl of anti-PHF serum were incubated (18 h, 4 °C) in 6 ml of a 1:2 homogenate of brain tissue in 50 mM Tris (pH 7.6)/0.15 M NaCl buffer (TS) containing 2 mM EDTA and 0.5 mM phenylmethylsulphonyl fluoride, yielding a 1:500 dilution of antiserum in absorbant. The absorbed supernatant was used as primary antiserum to stain TS-isolated tangles by the indirect peroxidase technique. To assess the tangle content of each absorbing homogenate, a 100-µl aliquot was heated in SDS/β-ME and pelleted (10,000g for 10 min). The resuspended pellet was placed on a slide and the content of tangles and neuritic plaque cores determined semi-quantitatively (0-4+) by light microscopy after Congo red staining. The intensity of tangle staining was reciprocally related to tangle content but not plaque core content of the absorbant.

* Purified PHF for immunization were obtained from this case.

activity of our serum was absorbed only by PHF-containing homogenates of AD cortex, not by tangle-free homogenates of AD and control brains (Table 1; Fig. 1D, E).

In an attempt to identify any normal antigens cross-reacting with our antiserum, unfractionated homogenates of AD and control brains were electrophoresed on SDS-polyacrylamide gels¹¹, electrophoretically transferred to nitrocellulose sheets¹² and stained with the antiserum at a dilution of 1:500 (Fig. 2A). In all tangle-containing AD homogenates, the band of excluded material (containing the PHF⁸) at the top of the gel was intensely labelled. No such band was stained in immunoblots of control homogenates. In AD but not control homogenates, the antiserum also stained a diffuse smear of material throughout the running gel (Fig. 2A). This smear of immunoreactive material was decreased if the AD homogenate was not boiled before sample loading; conversely, sonication and prolonged boiling accentuated it. Thus, the smear seems to represent immunoreactive material released from PHF-containing samples into the gel by heating and sonication. In both AD and control homogenates, three to four protein bands of MW between 35K and 60K were stained by the antiserum at 1:200 dilution, but were very faint or not visible at ≥1:500 dilution (Fig. 2A). Absorption with control homogenates eliminated the staining of these bands; the staining of the excluded band and smear in AD samples persisted. The latter staining was abolished if the serum was absorbed with PHF-rich AD cortex. Whether unabsorbed or control-absorbed serum was used, the serum consistently discriminated PHF-containing AD cortical homogenates from numerous aged and diseased control homogenates (Fig. 2A). The antiserum failed to label the neurofilament triplet proteins or any other brain cytoskeletal proteins (including tubulin and glial filament protein) from humans (Fig. 2A) or rabbits, even at a dilution of 1:100. Similarly, no cross-reactive proteins were detected in unfractionated homogenates of young or aged rhesus monkey brains, nor in human cerebrospinal fluid, serum or blood cells from numerous normal and AD cases.

Thus, the use of highly purified PHF as immunogen has produced an antiserum which reacts at high titre with: (1) a

large majority of neurofibrillary tangles in AD cortex; (2) almost all tangles purified by extraction and heating in SDS/β-ME and thus free of associated normal cytoskeletal elements; (3) tangles isolated in non-denaturing conditions (Fig. 1C); and (4) the high-molecular weight material containing PHF which is excluded from gels of AD samples. These anti-PHF antibodies seem to be specific for PHF and fail to react with normal brain fibrous proteins in tissue sections or on immunoblots. The proteins of other tissues tested to date also do not cross-react with the antibodies by immunoblotting, a highly sensitive technique for the immunochemical detection of protein antigens¹².

Previous studies have reported the staining of *in situ* tangles in AD tissue sections using either polyclonal^{10,13,14} or monoclonal^{15,16} antibodies that cross-react with one or more of the neurofilament triplet proteins. However, the absence of anti-neurofilament reaction using the antiserum described here led us to re-examine the tangle staining of some of these reagents. As reported above, the anti-200K rat neurofilament serum¹⁰ no longer stains tangles after their isolation in SDS/β-ME. Similarly, we have recently found that monoclonal antibodies to the 160K and 200K neurofilament proteins which stain tangles *in situ*¹⁵ fail to do so after SDS/β-ME extraction of the tangles¹⁷.

The most likely explanation for the findings reported here is that PHF represent a highly modified form of neurofilaments or another neuronal protein, presumably as a result of extensive post-translational processing. This hypothesis could explain why our antibodies are highly specific for determinants on PHF and do not recognize neurofilaments or other native brain proteins. Such extensive molecular modification is also consistent with our earlier observation of unusual chemical properties of PHF, for example, their high degree of insolubility in harsh denaturants^{8,9}. The neurofilament-cross-reactive material that is identified by antibodies to normal neurofilaments in tangles *in situ*^{10,13-16} could represent an intermediate (and still soluble) form of fibrous structure prior to formation of the final, insoluble PHF.

Identification of the specific antigens within PHF fibres that are recognized by our antibodies must await the complete

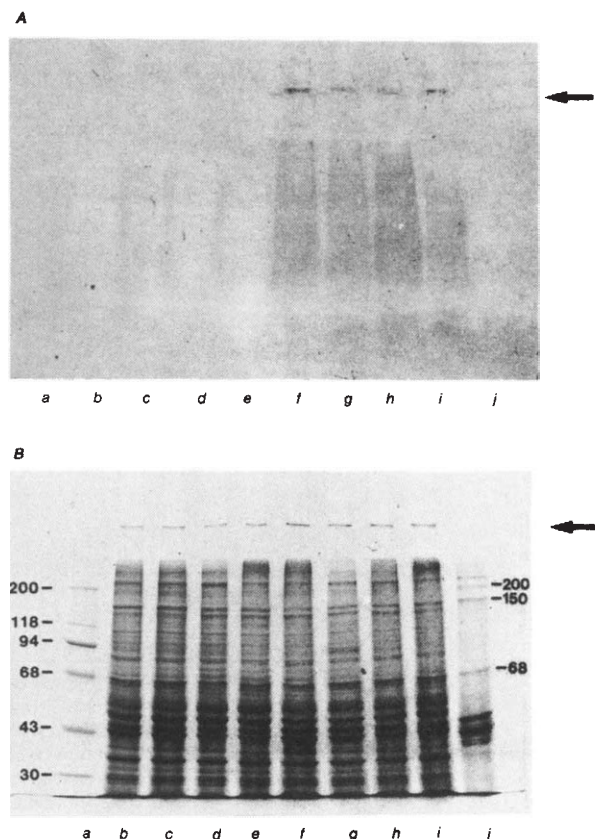


Fig. 2 *A*, Immunoblot of various control and AD whole cortical homogenates in Tris-saline buffer stained with anti-PHF antiserum (unabsorbed) at 1:500 dilution (indirect peroxidase method). *B*, Coomassie staining of identical gel to that blotted in *A*. Neuropathological diagnoses and ages (in years) of each case are as follows: *a*, MW standards; *b*, normal (2); *c*, normal (35); *d*, normal (92); *e*, normal (85); *f*, AD (75); *g*, AD (86); *h*, familial (autosomal dominant) AD (38); *i*, Down's syndrome with AD (49); *j*, human brain filament fraction (including neurofilament proteins of MWs 200K, 150K and 68K). Arrows indicate excluded bands in the sample wells at the top of the gel.

purification and compositional analysis of these insoluble fibres. However, available evidence is consistent with the notion that the PHF are composed of fibrous proteins. Our purified PHF preparations, including those used here as an immunogen, have a highly reproducible amino acid composition (C. G. Rasool, L. K. Duffy, C.A. and D.J.S., unpublished observations). The appearance of immunostained tangles with our antiserum is identical to their appearance with Bodian silver staining, a technique that has recently been shown to selectively stain neurofilament polypeptides¹⁸. The immunoreactive PHF-containing band on immunoblots (Fig. 2A) is intensely stained by Coomassie blue, a dye widely used for the detection and quantitation of proteins^{11,19}. Furthermore, SDS-extracted tangles can be directly stained by Coomassie blue to give a pattern identical to the immunostaining with our antiserum (C.A. and D.J.S., unpublished observations). Attempted staining of isolated tangles with reagents that detect carbohydrates (for example, concanavalin A and dansylhydrazine) does not give a positive reaction (C.A. and D.J.S., unpublished observations). These various findings, as well as the ultrastructure of PHF (twisted intermediate filaments), suggest that PHF are composed of fibrous proteins.

The availability of antibodies that specifically recognize PHF in human brain tissue should now allow immunoaffinity purification of PHF to homogeneity and subsequent detailed analysis of the composition and molecular origin of these abnor-

mal neuronal fibres in AD and other human neurofibrillary diseases.

Received 3 May; accepted 21 June 1983.

- Kidd, M. *Nature* **197**, 192-194 (1963).
- Terry, R. D., Gonatas, N. F. & Weiss, M. *Am. J. Path.* **44**, 269-297 (1964).
- Wisniewski, H. M., Narang, H. K. & Terry, R. D. *J. neurop. Sci.* **27**, 173-181 (1976).
- Tomlinson, B. E., Blessed, G. & Roth, M. *J. neurop. Sci.* **11**, 205-243 (1970).
- Ball, M. J. *Acta neuropath.* **37**, 111-118 (1977).
- Tomlinson, B. E. & Henderson, G. in *Neurobiology of Aging* (eds Terry, R. D. & Gershon, S.) 183-204 (Raven, New York, 1976).
- Perry, E. K., Tomlinson, B. E., Bergman, K., Gibson, P. H. & Perry, R. H. *Br. med. J.* **ii**, 1457-1459 (1978).
- Selkoe, D. J., Ihara, Y. & Salazar, F. J. *Science* **215**, 1243-1245 (1982).
- Selkoe, D. J., Abraham, C. & Ihara, Y. *Neurology* **33**, 35 (1983).
- Ihara, Y., Nukina, N., Sugita, H. & Toyokura, Y. *Proc. Japan. Acad.* **57**, 152-156 (1981).
- Laemmli, U. K. *Nature* **227**, 680-695 (1970).
- Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
- Gambetti, P. et al. in *Ageing of the Brain and Dementia* (eds Amaducci, L., Davison, A. N. & Antuono, A.) (Raven, New York, 1980).
- Dahl, D., Selkoe, D. J., Pero, R. T. & Bignami, A. *J. Neurosci.* **2**, 113-119 (1982).
- Anderton, B. H. et al. *Nature* **298**, 84-86 (1982).
- Autilio-Gambetti, L., Gambetti, P. & Crane, R. C. in *Alzheimer's Disease* (ed. Katzman, R.) (Cold Spring Harbor Laboratory, New York, in the press).
- Rasool, C. G., Anderton, B. H., Kahn, J., Ihara, Y. & Selkoe, D. J. *J. Neuropath. exp. Neurol.* **42**, 335 (1983).
- Gambetti, P., Autilio-Gambetti, L. & Papasozomenos, S. *Ch. Science* **213**, 1521-1522 (1983).
- Bradford, M. M. *Analyt. Biochem.* **72**, 248-254 (1976).

ATP excites a subpopulation of rat dorsal horn neurones

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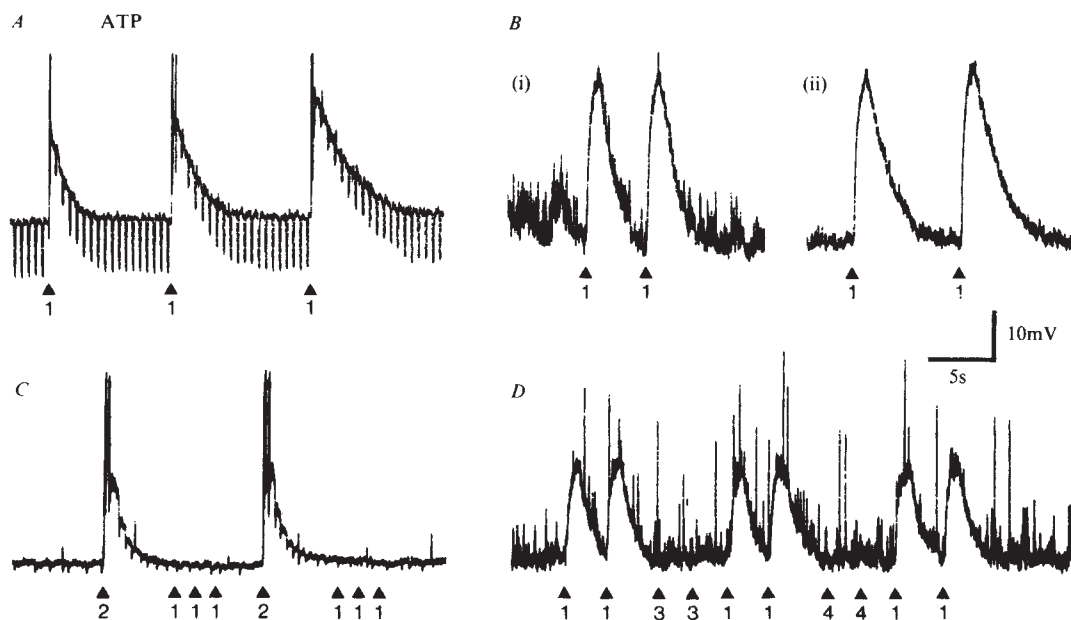
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The peripheral receptive properties and central projections of different classes of dorsal root ganglion neurones are well characterized^{1,2}. Much less is known about the transmitters used by these neurones. Excitatory amino acids have been proposed as sensory transmitters³ but the sensitivity of virtually all central neurones to those compounds has made it difficult to assess their precise role in sensory transmission. Several neuropeptides have been localized within discrete subclasses of primary sensory neurones that project to the superficial dorsal horn of the spinal cord⁴⁻⁷ and may be afferent transmitters. However, only about one-third of spinal sensory neurones have been shown to contain neuropeptides⁸. We have recently described the presence of a 5'-nucleotide hydrolysing acid phosphatase in a separate subpopulation of dorsal root ganglion neurones that project to the superficial dorsal horn⁴. This enzyme also appears in certain autonomic and endocrine cells that contain high concentrations of releasable nucleotides in their storage granules⁹⁻¹³. It is possible that the presence of this enzyme in sensory neurones is also associated with a releasable pool of nucleotides. Holton and Holton have provided evidence that ATP is released from the peripheral terminals of unmyelinated sensory fibres¹⁴ and have suggested that release of ATP might also occur from central sensory terminals. To investigate the possibility that nucleotides act as central sensory transmitters we have examined their actions on rat dorsal horn and dorsal root ganglion neurones maintained in dissociated cell culture. We report here a selective and potent excitation of subpopulations of both neuronal types by ATP.

Intracellular recordings were made from rat dorsal horn and dorsal root ganglion neurones grown in dissociated cell culture, separately or in co-culture for periods of 4-12 weeks. Micro-electrodes filled with 2 M potassium methylsulphate (100-200 MΩ) were used for intracellular recordings. Nucleotides and other drugs were applied to neuronal somata by pressure ejection from micropipettes with tip diameters of 4-6 μm, or by iontophoresis.

Pressure application of ATP (10^{-6} - 10^{-5} M) produced a rapid and marked depolarization (5-35 mV) in 27% (50 of 186) of

Fig. 1 Chart records of intracellular recorded responses of dorsal horn neurones to ATP. **A**, Disodium ATP (10^{-5} M) was ejected ($\blacktriangle$) by pressure (1.5 p.s.i.) from a micropipette positioned ~ 15 μ m from the soma of the recorded neurone in pulses of 50, 100 and 200 ms duration. The fast downward deflections were produced by injecting constant current hyperpolarizing pulses of 100 ms at 80 pA through the recording electrode and provide a measure of input resistance. Action potentials are truncated in this and all subsequent figures. Resting potential = -62 mV. **B(i)**, Depolarizations of another dorsal horn neurone caused by pressure ejection of 10^{-5} M ATP (0.5 s, 1 p.s.i.) in control recording medium (in mM; KCl, 5.36; KH_2PO_4 , 0.44; MgCl_2 , 0.49; MgSO_4 , 0.41; NaCl, 136.9; Na_2HPO_4 , 0.34; CaCl_2 , 3.26; glucose, 50; 1% heat inactivated rat serum; 0.2% phenol red). **B(ii)**, after the addition of 100 μ M CdSO_4 which blocked all spontaneous postsynaptic potentials (seen in **B(i)** as random fast upward deflections). Resting potential = -64 mV. **C**, A dorsal horn neurone which was unresponsive to ATP (10^{-5} M; 1 s, 1.5 p.s.i.; applied at triangles labelled '1') was strongly excited by L-glutamate (10^{-4} M; 0.1 s, 1.5 p.s.i.; applied at '2'). Fast downward deflections were spontaneous postsynaptic potentials. Resting potential = -66 mV. **D**, A dorsal horn neurone which was responsive to ATP (10^{-5} M; 1 s, 1.5 p.s.i.; applied at triangles labelled '1') but not adenosine (10^{-4} M; 1 s, 1.5 p.s.i.; applied at '3') or GTP (10^{-4} M; 1 s, 1.5 p.s.i.; applied at '4'). Fast upward deflections were spontaneous postsynaptic potentials and action potentials. Resting potential = -59 mV. Calibration bar applies to all records. All records in Figs 1 and 2 are from neurones derived from the dorsal half of spinal cords from 15-day rat embryos. After removal of the dura, the spinal cord tissue was dissociated with trypsin and DNase as previously described³⁰. The resulting cell suspension was dispensed onto collagen-coated 35 mm tissue culture dishes containing a monolayer of non-neuronal cells (mainly astrocytes derived from 15–17-day embryonic rat spinal cord).



dorsal horn neurones tested (Fig. 1A). The response to ATP was often accompanied by one or more action potentials superimposed on the depolarization. The remaining neurones were unaffected by ATP (even at a concentration of 10^{-4} M; seven of seven neurones tested), whereas 96% (65 of 68) of dorsal horn neurones were strongly excited by glutamate (10^{-5} – 10^{-4} M; Fig. 1C). The majority of dorsal horn neurones received spontaneous synaptic input. Superfusion with CdSO_4 (100 μ M) completely abolished all spontaneous synaptic activity but did not decrease the response to ATP, indicating that the depolarization elicited by ATP was the result of a direct action on the membrane of neurones from which recordings were made (Fig. 1B). The disodium, magnesium, calcium and Tris salts of ATP were equally effective in depolarizing dorsal horn neurones. Furthermore, the chelators EDTA (10^{-5} M) and inorganic pyrophosphate (10^{-4} M) were without effect. It is unlikely, therefore, that the actions of ATP are mediated by the chelation of divalent cations¹⁵.

Depolarization of dorsal horn neurones was observed only with ATP and closely related analogues. Adenosine (Fig. 1D), AMP, GTP (Fig. 1D) and UTP were without effect while ADP and CTP were less than 1/10th as active as ATP. Adenosine tetraphosphate, which is not an effective substrate for ATPases, and the slowly hydrolysable ATP analogues, AMP-PNP and β - γ -methylene ATP, also depolarized dorsal horn neurones, indicating that the ATP-induced excitation of dorsal horn neurones is unlikely to require hydrolysis of the triphosphate chain. Superfusion of dorsal horn neurones with 8-phenyltheophylline at a concentration sufficient to block adenosine receptors¹⁶ did not decrease the depolarization produced by ATP. This provides further evidence that the response of dorsal horn neurones to ATP does not involve activation of adenosine receptors¹⁷. The ATP-evoked excitation of dorsal horn neurones was also unaffected by isatogen derivatives that have been reported to antagonize ATP responses on smooth muscle preparations¹⁸.

The depolarization of dorsal horn neurones was accompanied by an increase in membrane conductance that persisted when the membrane potential was maintained near the resting level by injection of current through the recording electrode (Fig. 2A). The increase in conductance is therefore due to an action of ATP itself and did not result from voltage-dependent membrane rectification. ATP depolarized neurones that were hyperpolarized by application of γ -aminobutyric acid (GABA, Fig. 2B) which has been shown to increase chloride conductance of cultured spinal neurones¹⁹. It is, therefore, unlikely that ATP excites dorsal horn neurones by opening chloride channels. To evaluate the contribution of sodium ions, we examined the effect of altering the extracellular sodium concentration on the depolarization elicited by ATP. Reducing the extracellular sodium concentration to 7.5 mM abolished the excitatory action of ATP (Fig. 2C). On the same neurone, the excitation evoked by L-glutamate, which is probably mediated by an increase in sodium conductance²⁰, was also abolished. These results suggest that the depolarization of dorsal horn neurones by ATP is due primarily to an increase in sodium conductance. Application of tetrodotoxin at 10^{-6} M blocked all regenerative activity but did not affect the ATP response. The conductance increase produced by ATP cannot, then, be a consequence of opening the voltage-dependent channels involved in the propagation of action potentials. Recent reports have described excitatory actions of ATP on *Xenopus* oocytes²¹, molluscan neurones²², sympathetic neurones^{23,24}, parotid acinar cells²⁵ smooth muscle of the guinea pig vas deferens²⁶ and pancreatic β -cells^{27–30}. However, the action of ATP on these cells seems to involve ionic mechanisms that differ from that underlying the depolarization of dorsal horn neurones.

Since sympathetic neurones and pancreatic islet cells reported to be sensitive to ATP contain the nucleotide-hydrolysing acid phosphatase enzyme found in sensory neurones, we have also examined the nucleotide sensitivity of primary sensory neurones. The majority of rat dorsal root ganglion neurones

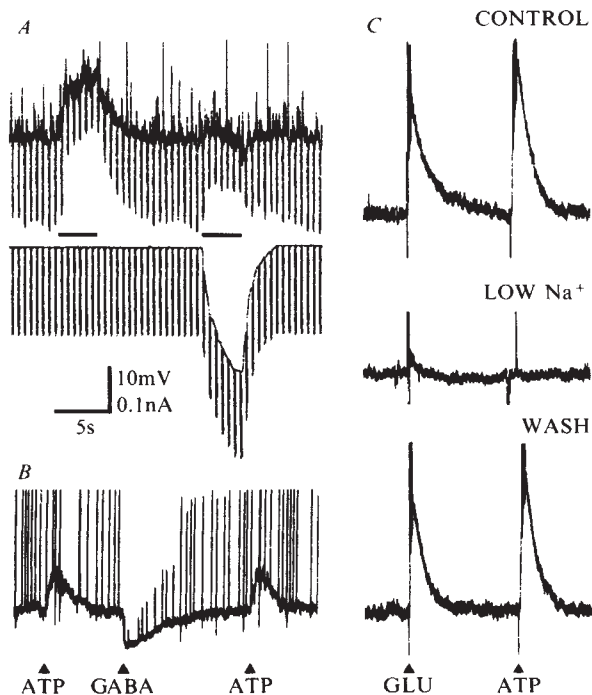


Fig. 2 The ionic dependence of ATP-evoked depolarization of dorsal horn neurones. **A**, (Upper trace), chart record of intracellular voltage responses produced by two identical pressure applications of ATP (10^{-5} M; 3 s, 2 p.s.i.; marked by bars) from a pipette tip positioned 100 μ m from the dorsal horn neurone (to decrease the rate of rise of the response). During the second application, the voltage was held near to the resting membrane potential by manually adjusting the amount of current injected through the recording microelectrode. The current is monitored in the lower trace. Resting potential = -55 mV. **B**, When another dorsal horn neurone was depolarized to -48 mV by injecting positive d.c. current through the recording electrode the response to ATP (10^{-5} M; 0.4 s, 2 p.s.i.) was depolarizing while GABA (10^{-4} M; 0.1 s, 2 p.s.i.) hyperpolarized the cell. Before and after current injection the resting potential was -62 mV and the responses to both ATP and GABA were depolarizing. **C**, Responses of another dorsal horn neurone to iontophoresis of Tris glutamate (100 mM; pH 8; 0.3 s, 5 nA) and Mg^{2+} -ATP (0.4 M; pH 5; 0.5 s, 5 nA except in the middle trace where the duration was 0.8 s) in control medium (Control), after switching to medium containing 7.5 mM Na^{+} (Low Na^{+} ; sodium chloride was replaced by sucrose, 260 mM) and after returning to control medium (Wash). $CdCl_2$ (200 μ M) was present in all conditions in order to block synaptic transmission. Identical results were obtained when NaCl was replaced by equimolar choline chloride. In sodium substitution experiments, ATP was applied by iontophoresis because pressure application transiently changes the extracellular environment of the neurone under study to that inside the pipette. This precludes the use of the same pipette in control and experimental conditions. In contrast, the iontophoretic ejection of ATP is not associated with significant alterations in extracellular sodium concentration. Only those cells that responded to ATP applied by pressure were found to respond to iontophoretic application of ATP. The converse was also true

(91 of 102) in culture were depolarized by ATP, however, the remaining cells were unresponsive even at high (10^{-4} M) ATP concentrations. The pharmacological specificity of nucleotides tested on sensory neurones was essentially the same as that of dorsal horn neurones. The depolarization of sensory neurones was also associated with an increase in membrane conductance which was not attributable to voltage-dependent membrane rectification (Fig. 3A). The response to ATP was abolished after substitution of 90% of the extracellular sodium with choline, indicating that depolarization of sensory neurones is also due primarily to an increase in sodium conductance.

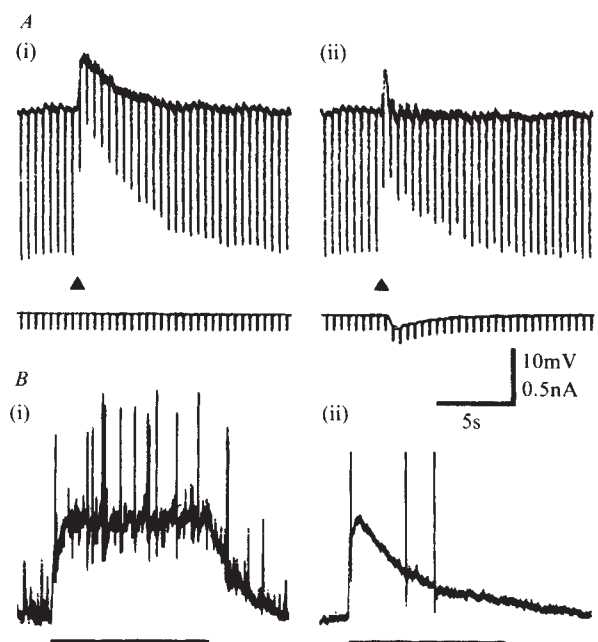


Fig. 3 Depolarization and desensitization produced by ATP in dorsal root ganglion neurones. **A** (i), Upper trace shows the depolarization and conductance increase produced by pressure ejection of ATP (10^{-5} M; 0.2 s, 2 p.s.i.). The lower trace monitors the current passed through the recording microelectrode. (ii), Response to an identical pulse of ATP to that in (i). Here the membrane potential was held constant by changing the amount of current passed through the recording microelectrode. The current is monitored in the lower trace. Resting potential, -60 mV. **B**, The response of a dorsal root ganglion neurone to a 10-s pulse of ATP (10^{-5} M; 1.5 p.s.i.). Resting potential, -58 mV. (ii) A 10-s pulse of ATP (10^{-5} M; 1.5 p.s.i.) applied to a dorsal root ganglion neurone was associated with a marked desensitization to ATP. Resting potential, -56 mV. Dorsal root ganglia from 1-3 day postnatal rats were dissociated and plated as previously described³⁰.

Although the ATP-induced excitation of both cell types was similar, we observed that prolonged application of ATP to dorsal horn neurones produced a sustained depolarization (Fig. 3B(i)) whereas the response of sensory neurones was frequently associated with a rapid desensitization of both the depolarization (Fig. 3B(ii)) and the conductance increase. In some cells the desensitization could last for minutes. Dorsal root fibres in isolated spinal cord preparations are also depolarized by ATP³¹ indicating that the sensitivity of neurones to ATP is not restricted to the cell body. If the depolarization and desensitization reported here also occur at the terminals of sensory neurones *in vivo*, these events could modify the transmission of sensory information.

Our results provide evidence that subpopulations of sensory and dorsal horn neurones that are likely to be involved in the processing and regulation of sensory stimuli are potently and selectively excited by ATP. In dissociated cell culture, the identification and origin of dorsal horn neurones that respond to ATP are unclear. *In vivo*, ATP has been reported to increase the firing rate of cuneate neurones that have cutaneous and proprioceptive input¹⁵ and to activate both nociceptive and non-nociceptive units in the dorsal horn of the medulla³². Central neurones with sensory input are therefore excited by ATP *in vivo*. In the spinal cord, the excitatory action of ATP may be restricted to neurones in the dorsal horn since ATP does not appear to excite neurones in the ventral horns of cat³³ toad³¹ or newborn rat (K. Yoshioka, unpublished observations).

There is now considerable information on the possible transmitter actions of ATP in the central and peripheral nervous system^{4,26,34-36}. The most convincing evidence for a sensory transmitter role of ATP has been provided by Holton and her

colleagues¹⁴. ATP mimics the vasodilation that follows antidromic activation of nociceptive sensory fibres and the effect of ATP is blocked by drugs that abolish nerve-evoked vasodilation³⁶. Moreover, ATP is released into the circulation following peripheral nerve stimulation³⁷. Although at present there are no anatomical techniques for identifying individual sensory neurones that might release ATP, the dependence of antidromic vasodilation on activation of unmyelinated fibres³⁸ suggests that this class of afferents is most likely to be responsible. Since ATP is present in high concentrations in the storage vesicles of many cells³⁹ including neuronal and endocrine cells that contain a nucleotide hydrolysing acid phosphatase⁴⁴, we are interested in the possibility that this enzyme is present in sensory neurones that release nucleotides.

While the present studies have focused on the actions of nucleotides, substance P has also been implicated as a transmitter at the central and peripheral terminals of small sensory neurones^{40,41}. The availability of effective substance P⁴² and ATP^{26,43} receptor antagonists may allow a critical assessment of the role of these substances as sensory transmitters.

We thank Drs J. Dodd, E. Frank, J. P. Horn and P. MacLeish for helpful comments. Professor M. Hooper kindly provided the isotogen derivatives. This work was supported by grants to T.M.J. from the NIH (NS17368), the Dysautonomia Foundation, the Muscular Dystrophy Association and the McKnight Foundation. C.E.J. was supported by NIH postdoctoral training grants NS07112 and NS07051.

Note added in proof: Krishtal *et al.* have recently reported an ATP-activated sodium conductance in neurones from a variety of sensory ganglia⁴⁵.

Received 21 March; accepted 21 June 1983.

- Burgess, P. R. & Perl, E. R. in *Handbook of Sensory Physiology* Vol. II (ed. Iggo, A.) 29–78 (Springer, New York, 1973).
- Brown, A. G. *Organization in the Spinal Cord* (Springer, New York, 1981).
- Curtis, D. R. & Johnston, G. A. R. *Ergebn. Physiol.* **69**, 97–188 (1974).
- Hökfelt, T. *Neurosci. Res. Program Bull.* **17**, 425–443 (1979).
- Fuxe, K., Ganten, D., Hökfelt, T. & Bolne, P. *Neurosci. Lett.* **2**, 229–234 (1976).
- Dalsgaard, C. J. *et al. Neurosci. Lett.* **33**, 159–163 (1982).
- Panula, P., Hadjiconstantinou, M., Yang, H. Y. T. & Costa, E. *Soc. Neurosci. Abstr.* **8**, 135 (1982).
- Jessell, T. M. in *Brain Peptides* (eds Krieger, D. T. *et al.*) (Wiley, New York, in the press).
- Smith, A. D. & Winkler, H. *Handbk exp. Pharmac.* **33**, 538–617 (1972).
- Stjarne, L. *Handbk exp. Pharmac.* **33**, 231–269 (1972).
- Von Euler, U., Lishajko, F. & Stjarne, L. *J. Acta physiol. scand.* **59**, 495–496 (1963).
- Leitner, J. W., Sussman, K. E., Vatter, A. E. & Schneider, F. H. *Endocrinology* **95**, 662–677 (1975).
- Douglas, W. H. & Poisner, A. M. *J. Physiol., Lond.* **183**, 249–256 (1966).
- Holton, F. A. & Holton, P. J. *J. Physiol., Lond.* **126**, 124–140 (1954).
- Galindo, A., Krnjevic, K. & Schwartz, S. J. *Physiol., Lond.* **192**, 359–377 (1967).
- Furshpan, E. J., Potter, D. D. & Landis, S. C. *Harvey Lect.* **76**, 149–191 (1981).
- Burnstock, G. *J. theor. Biol.* **62**, 491–503 (1976).
- Hooper, M., Spedding, M., Sweetman, B. J. & Weetman, D. F. B. *J. Pharmac.* **50**, 458P–459P (1974).
- Barker, J. L. & Ransom, B. R. *J. Physiol., Lond.* **280**, 331–354 (1978).
- Sonnhof, V. & Bührle, Ch. in *Glutamate as a Neurotransmitter* (eds DiChiari, G. & Gess, G. L.) 195–204 (Raven, New York, 1981).
- Lotan, L., Dascal, N., Cohen, S. & Lass, Y. *Nature* **298**, 572–574 (1982).
- Yatani, A., Tsuda, Y., Akaike, N. & Brown, A. M. *Nature* **296**, 169–171 (1982).
- Siggins, G. R., Gruol, D. L., Padien, A. L. & Forman, D. S. *Nature* **270**, 263–265 (1982).
- Akasu, T., Hirai, K. & Koketsu, K. *Brain Res.* **258**, 313–317 (1983).
- Gallagher, D. V. *Nature* **296**, 83–86 (1982).
- Sneddon, P., Westfall, D. P. & Fedan, J. S. *Science* **218**, 693–695 (1982).
- Loubatiers, A. L., Loubatiers-Mariani, M. M. & Chapal, J. C. *R. Soc. Biol. Acad. Sci., Paris* **166**, 1742–1746 (1972).
- Wier, G. C., Knowlton, St D. & Martin, D. B. *Endocrinology* **97**, 932–936 (1975).
- Chapal, J. & Loubatiers-Mariani, M. M. *Br. J. Pharmac.* **73**, 105–110 (1981).
- Yamamoto, M., Steinbusch, H. M. W. & Jessell, T. M. *J. Cell Biol.* **91**, 142–152 (1981).
- Phillips, J. W. & Kirkpatrick, J. R. *Gen. Pharmac.* **9**, 239–247 (1978).
- Salt, T. E. & Hill, R. G. *Neurosci. Lett.* **35**, 53–57 (1983).
- Curtis, D. R., Phillis, J. W. & Watkins, J. C. *J. Physiol., Lond.* **158**, 296–323 (1961).
- Stone, T. W. *Neuroscience* **6**, 523–555 (1981).
- Burnstock, G. *J. Physiol., Lond.* **313**, 1–35 (1981).
- Holton, P. J. *J. Physiol., Lond.* **120**, 95–104 (1953).
- Holton, P. J. *J. Physiol., Lond.* **145**, 494–504 (1959).
- Celander, O. & Folkow, B. *Acta physiol. scand.* **29**, 359–370 (1953).
- Poisner, A. M. & Trifaro, J. M. *The Secretory Granule* (Elsevier, Amsterdam, 1982).
- Otsuka, M. & Konishi, S. *Cold Spring Harb. Symp. quant. Biol.* **40**, 135–143 (1976).
- Lembeck, F. & Gamse, R. *Ciba Fdn Symp.* **91**, 35–54 (1982).
- Rosell, S., Olgart, L., Grazilius, B., Panopoulos, P., Folkers, K. & Hong, J. *Acta physiol. scand.* **111**, 381–383 (1981).
- Fedan, J. S., Hogaboam, G. K., Westfall, D. P. & O'Donnell, J. P. *Eur. J. Pharmac.* **81**, 193–204 (1982).
- Dodd, J. *et al. Cold Spring Harb. Symp. quant. Biol.* **48** (in the press).
- Krishtal, O. A., Marchenko, S. M. & Pidoplichko, V. I. *Neurosci. Lett.* **35**, 41–45 (1983).

Cyclic GMP stimulation of a light-activated ATPase in rod outer segments

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Rod outer segments (ROSs) of vertebrate photoreceptor cells have been reported to contain several enzyme systems including a dark, Ca^{2+} -stimulated ATPase¹, a rhodopsin kinase^{2,3}, a phosphodiesterase^{4,5} and a GTPase^{6,7}, all of which are light-stimulated. Recently, Thacher has found a light-stimulated Mg^{2+} -ATPase in frog ROSs^{8,9} while our own laboratory has identified a dark, Ca^{2+} -inhibited Mg^{2+} -ATPase in bovine ROSs^{10–12}. Here we extend our observations on the Mg^{2+} -ATPase and demonstrate that flash illumination following the dark ATPase process stimulated ATPase activity at a rate considerably faster than the dark process. In addition, we find that both the dark and light stimulated ATPase activities are markedly enhanced by cyclic GMP and inhibited by Ca^{2+} .

Bovine ROSs were prepared as intact stacks of disks ensheathed by a perforated plasma membrane¹³ and washed free of endogenous nucleotides. The ROSs were stored in ampoules at liquid N_2 temperature, which produced a ~20% reduction in amplitude of the scattering signals relative to freshly prepared ROSs but in no way altered the qualitative appearance of the signals. ATPase activity was monitored as the time course of the appearance of inorganic phosphate as well as the change in scattered light intensity ($-\Delta I/I$ at 810 nm) from dilute ($1 \mu\text{M}$ rhodopsin) ROS suspensions both in the dark before flash actinic illumination (A_D) and following the flash (A_L) (the flash bleached ~25% of the rhodopsin).

Figures 1 and 2 show the time courses of the relative scattered light intensity and the appearance of inorganic phosphate. After allowing the dark ATPase mediated process (A_D) to proceed for several minutes, subsequent flash actinic illumination produced an initial very rapid change in relative scattered light intensity ($\Delta I/I$) at an angular resolution of 10° (measured as an increase in $-\Delta I/I$) followed by a slower change (Fig. 3a). This overall light induced change has been designated A_L (ref. 13). The initial very rapid change in A_L is partly due to the hypsochromic (blue) shift in the anomalous dispersion spectrum associated with the photobleaching of rhodopsin to a mixture of metarhodopsins I and II¹³. It should be stressed that $\Delta I/I$ is strongly dependent on the angle at which the scattered light is measured. Thus, both A_D and A_L show an increase in $-\Delta I/I$ at 10° but a decrease at 0° . A narrow angular resolution ($\pm 1^\circ$) is therefore essential for proper observation of these signals.

The slow component in A_L can essentially be eliminated if after A_D and before flash illumination either hexokinase and glucose is added to the ROS suspension, thereby destroying any ATP, or if the ATP analogue AMP-PNP is added to block ATPase activity¹⁴ (Fig. 3a). This implies that a hydrolysis of the terminal phosphate on ATP is involved in the enzyme action and eliminates the possibility that activation of a cyclase enzyme present in ROSs¹⁵ is responsible for the observed signals.

Of significance in Fig. 3b is the observation that incubation of the ROS suspension with phosphodiesterase inhibitors before flash illumination had no effect on the $-\Delta I/I$ -time profile. Light activated rhodopsin kinase activity² is also ruled out as responsible for the A_L signals as rhodopsin phosphorylation is approximately 300 times slower than the observed rate of A_L (refs 13, 16). Calcium ion at concentrations 1/10th that of Mg^{2+} were found to inhibit both A_D and A_L by as much as 30%. When GTP replaces ATP, a signal similar in appearance to A_L is

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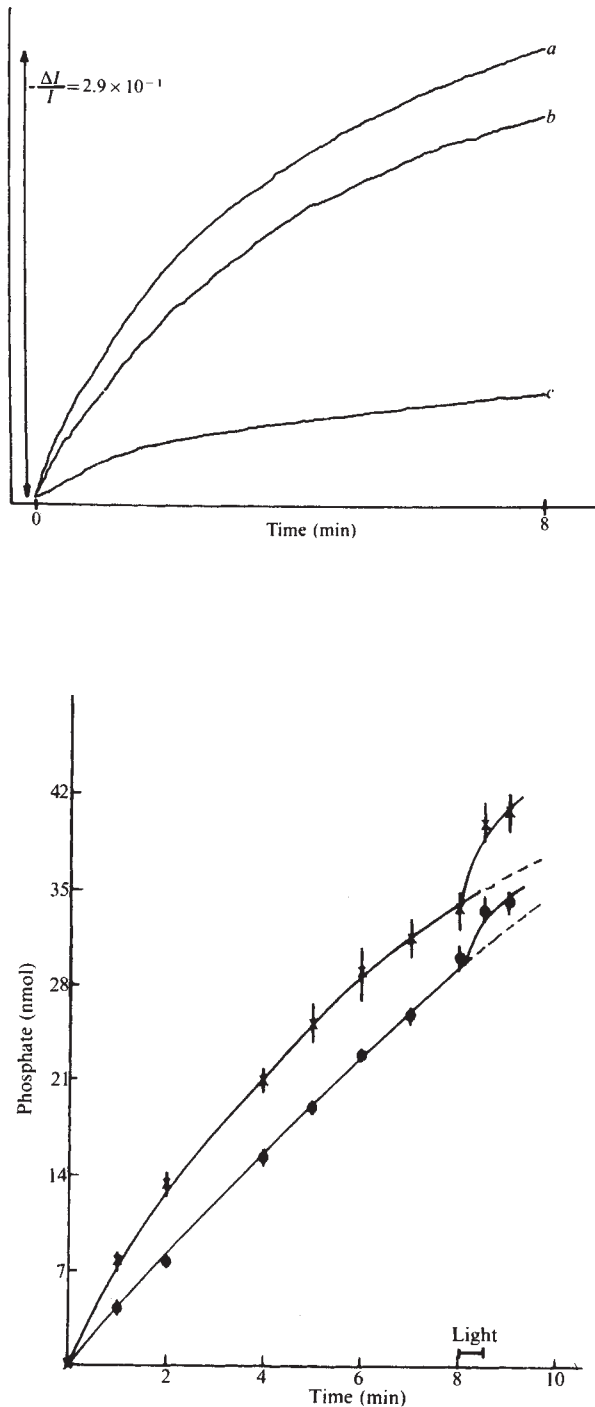


Fig. 2 Bovine ROS suspensions at 21 °C in 60 mM Tris-HCl pH 7.2, 2 mM MgCl_2 and 900 μM ATP, in the absence (circles) and presence (crosses) of 500 μM cyclic GMP. Aliquots of the suspension were removed at given times and the reaction was stopped with 11% trichloroacetic acid. The protein was separated by centrifugation and the supernatant analysed for inorganic phosphate by addition of Fe-ammonium molybdate¹⁸ and the optical density obtained at 750 nm. After 8 min in the dark the remaining ROS was bleached with white light for 20 s. Bleaching was done through a heat filter and no detectable ($<0.1^\circ\text{C}$) rise in temperature was observed. The same results were obtained in the presence of 0.1 mM ouabain, indicating that no ($\text{Na}^+ + \text{K}^+$) ATPase contamination had occurred. Error bars represent that standard error from three separate experiments without cyclic GMP and four experiments with cyclic GMP from the same bovine ROS preparation. Similar rates of phosphate appearance were observed in each of five different bovine ROS preparations. In the absence of exogenously added ATP no change in phosphate levels from time 0 could be detected over a 10 min period.

Fig. 1 Light-scattering changes ($\Delta I/I$) plotted as a function of time for a suspension of bovine ROSs at a concentration of 1 μM rhodopsin in 60 mM Tris-HCl pH 7.2, 1.3 mM MgCl_2 . *a*, Suspension made 1.6 mM in ATP and 100 μM cyclic GMP; *b*, suspension made 1.6 mM in ATP, no added cyclic GMP; *c*, no exogenously added ATP. Scattered light at 810 nm was measured at an angle $10^\circ \pm 1^\circ$ to the transmitted light. Temperature was 21 °C. In all experiments, ROSs were put into the cuvette under dim red light, an operation which took less than 30 s. For the rest of the incubation time the room was completely dark except for the monitoring light beam (810 nm).

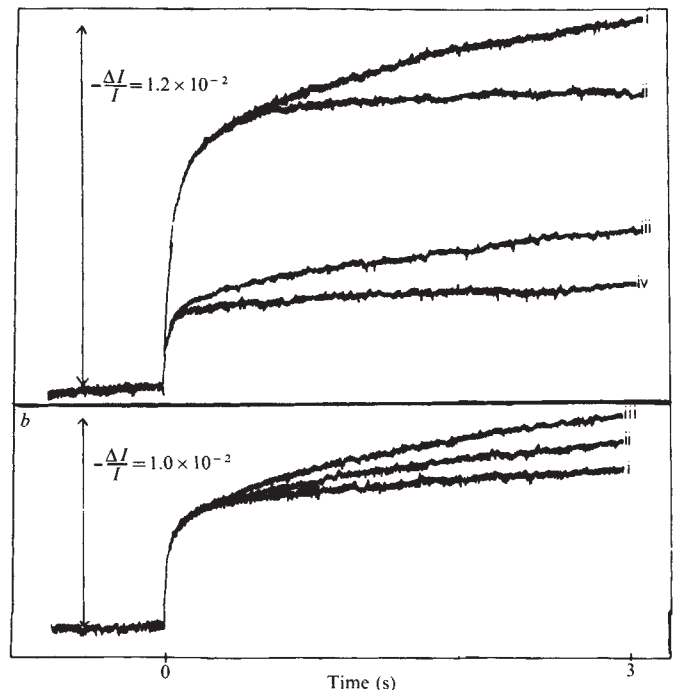


Fig. 3 Light scattering transients ($\Delta I/I$) as a function of time elicited from bovine ROS suspensions (1 μM rhodopsin) by flash illumination with actinic light at time 0 s. Each flash bleached $\sim 25\%$ of the rhodopsin. Scattered light 810 nm was monitored at a scattering angle $10^\circ \pm 1^\circ$ from the transmitted light at room temperature (21 °C). ROSs were suspended in 60 mM Tris-HCl pH 7.2, 1.3 mM MgCl_2 and 0.1 mM ouabain. *a* i, Signal elicited by flash illumination after 3 min incubation in the dark with 800 μM ATP. ii Represents four separate experiments that give identical results. In each experiment the solution was first made up identically to that of i and allowed to incubate for 3 min. The following experiments were then performed: (1) 30 U of hexokinase were added and the suspension was made 1 mM in glucose, allowing 1 min reaction time to deplete the remaining ATP before flashing at time 0 s. (2) Suspension made 600 μM in AMP-PNP and flashed. (3) Suspension made 10 μM in vanadate and flashed. (4) Suspension made 1.3 mM in CaCl_2 and flashed. iii, The signal observed when ATP was replaced with 800 μM GTP, dark incubated and flash illuminated. iv, The flash induced signal obtained from ROS suspensions when no nucleotides (ATP nor GTP) were added exogenously. *b*, Light scattering changes obtained on flash illumination after 3 min of dark incubation in 800 μM ATP. i, No cyclic GMP added exogenously. The same signal was obtained with either 5 mM isobutylmethylxanthine or 125 μM papaverine. Also, up to 500 μM cyclic AMP did not alter the signal obtained. ii, A suspension made 20 μM in cyclic GMP in addition to the ATP added. iii, A suspension made 160 μM in cyclic GMP and flashed.

observed but it is much smaller in amplitude (Fig. 3a), with very different properties, and has been shown to be due to another enzyme system¹³.

The above results, together with the observed enhanced inorganic phosphate release (Fig. 2), indicate the presence of a dark ATPase whose activity is considerably enhanced by rhodopsin photolysis. That these results might be due to inner segment ($\text{Na}^+ + \text{K}^+$) ATPase and/or mitochondrial contamination is ruled out by the fact that incubation with ouabain (0.1 mM for 5 min) or with the mitochondrial uncoupler SF6847 (ref. 17) (6×10^{-9} – 1×10 M) had no effect on A_D or A_L .

A most significant observation regarding the slow component of A_L is that the addition of micromolar quantities of cyclic GMP (but not cyclic AMP) markedly enhance this signal (Fig. 3b). This can be attributed to an increase in ATPase activity because light-stimulated inorganic phosphate release, as well as enhanced phosphate release in the dark incubation, were also observed in the presence of cyclic GMP (Fig. 2). The presence of cyclic GMP in the absence of Mg^{2+} -ATP elicited neither an A_D nor an A_L signal.

It should be stressed that the cyclic GMP enhancement effect for dark adapted bovine ROSs from young (~2.5 yr) heifers is substantial and reproducible. In the case of older and partially light adapted cows, however, the effect is very much reduced and erratic. It is difficult to make any quantitative statement concerning the rates of the cyclic GMP enhanced ATPase activity (Figs 2, 3) as both the rates and the saturation levels seem to change. This points to a complex role for cyclic GMP.

A significant point here is that when ROSs are prepared in the same manner as described above¹³ except that the plasma membrane is kept intact, the intracellular contents or at least the nucleotides are assumed to be retained within the ROSs, in contrast to perforated plasma membrane ROS preparations in which the nucleotides are washed out. With such intact plasma membrane preparations, without addition of exogenous ATP, we have observed light scattering signals quite comparable to those in which exogenous Mg^{2+} -ATP is added. Furthermore, when the plasma membranes of such preparations are perforated and treated with hexokinase and glucose to remove ATP, no A signals are observed. This suggests that the effects of Mg^{2+} -ATP on ROS preparations described above are physiological and not induced by the treatment used or additions of nucleotides to the ROS suspensions.

Thus, we have demonstrated the physiological presence of an ATPase in ROSs presumably located in the disk membranes. This ATPase is active in the dark and is stimulated to further, more rapid activity on flash photolysis of rhodopsin. Both dark and light ATPase activities are inhibited by Ca^{2+} at concentrations much lower than those of Mg^{2+} and are enhanced by cyclic GMP. As cyclic GMP is consumed in the light reaction^{4,5}, its regulatory function in both dark and light induced ATPase activity suggests a link between ATPase and phosphodiesterase activity.

This work was supported by a research grant to E.W.A. from the National Research Council of Canada. We thank A. Geisterfer and R. Jones for aid in the phosphate assays and in preparation of the manuscript.

Received 18 January; accepted 13 June 1983.

- Sack, R. & Harris, C. *Nature* **265**, 465–466 (1977).
- Kühn, H. & Dreyer, W. *FEBS Lett.* **20**, 1–6 (1972).
- Bownds, D., Dawes, J., Miller, J. & Stahlman, M. *Nature new Biol.* **237**, 125–127 (1972).
- Miki, N., Baraban, J. M., Keirns, J. J., Boyce, J. J. & Bitensky, W. J. *J. biol. Chem.* **250**, 6320–6327 (1975).
- Keirns, J. J., Miki, N., Bitensky, M. & Keirns, M. *Biochemistry* **14**, 2760–2765 (1975).
- Robinson, W. E. & Hagins, W. A. *Biophys. J.* **17**, 196a (1977).
- Wheeler, G. L. & Bitensky, M. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4238–4242 (1977).
- Thacher, S. *Biophys. J.* **21**, 136a (1978).
- Thacher, S. *Biochemistry* **17**, 3005–3011, (1978).
- Uhl, R., Borys, T. & Abrahamson, E. W. *Biophys. J.* **21**, 136a (1978).
- Uhl, R., Borys, T. & Abrahamson, E. W. *Photochem. Photobiol.* **29**, 703–706 (1979).
- Uhl, R., Borys, T. & Abrahamson, E. W. *FEBS Lett.* **107**, 317–322 (1979).
- Borys, T. J. thesis, Univ. Guelph (1981).
- Yount, R., Babcock, D., Ballantyne, W. & Ojala, D. *Biochemistry* **10**, 2484–2489 (1971).
- Fleischman, D. & Denisevich, M. *Biochemistry* **18**, 5060–5066 (1979).
- Kühn, H. & Bader, S. *Biochim. biophys. Acta* **428**, 13–18 (1976).
- Nishizawa, Y., Sumido, S. & Murakami, S. *Am. chem. Soc. Symp. Ser.* **2**, 169 (1974).
- Tausky, H. & Schorr, E. *J. biol. Chem.* **202**, 675 (1953).

Oscillations of intracellular Ca^{2+} in mammalian cardiac muscle

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Contraction of cardiac muscle depends on a transient rise of intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) which is initiated by the action potential¹. It has, however, also been suggested that $[\text{Ca}^{2+}]_i$ can fluctuate in the absence of changes in membrane potential. The evidence for this is indirect and comes from observations of (1) fluctuations of contractile force in intact cells^{2–5}, (2) spontaneous cellular movements⁶, and (3) spontaneous contractions in cells which have been skinned to remove the surface membrane⁷. The fluctuations in force are particularly prominent when the cell is Ca^{2+} -loaded, and have been attributed to a Ca^{2+} -induced Ca^{2+} release from the sarcoplasmic reticulum⁷. In these conditions of Ca^{2+} -loading the normal cardiac contraction is followed by an aftercontraction⁸ which has been attributed to the synchronization of the fluctuations⁵. The rise of $[\text{Ca}^{2+}]_i$ which is thought to underlie the aftercontraction also produces a transient inward current³. This current, which probably results from a Ca^{2+} -activated nonspecific cation conductance⁹, has been implicated in the genesis of various cardiac arrhythmias. However, despite the potential importance of such fluctuations of $[\text{Ca}^{2+}]_i$, their existence has, so far, only been inferred from tension measurements. Here we present direct measurements of such oscillations of $[\text{Ca}^{2+}]_i$.

The experiments were performed on ferret papillary muscles which were microinjected with the photoprotein aequorin in order to measure $[\text{Ca}^{2+}]_i$. In most experiments between 30 and 100 cells were injected. The intensity of the light emitted by aequorin (a function of $[\text{Ca}^{2+}]_i$) was measured with a photomultiplier tube which gave a current proportional to light intensity (details are given in the figure legends, see also ref. 10). In the first set of experiments we investigated the effects, on the twitch and accompanying Ca^{2+} transient, of manoeuvres which elevate the resting $[\text{Ca}^{2+}]_i$. The most consistent method found to elevate $[\text{Ca}^{2+}]_i$ was to replace external Na^+ with another monovalent cation. The increase of $[\text{Ca}^{2+}]_i$ is attributable to the effects of Na^+ removal on the Na^+ - Ca^{2+} exchange¹¹. Replacement of Na^+ with lithium (Fig. 1A, b) increased both resting $[\text{Ca}^{2+}]_i$ and the amplitude of the calcium transient. More interestingly, following the calcium transient, $[\text{Ca}^{2+}]_i$ fell below the initial level and then rose again, after which it returned to the resting level in a series of damped oscillations. Resting $[\text{Ca}^{2+}]_i$ fell on longer exposure to zero Na^+ solution (Fig. 1A, c). This was accompanied by a decrease in both the amplitude and frequency of the oscillations of $[\text{Ca}^{2+}]_i$. Figure 1B shows, in agreement with previous work¹², that similar oscillations can be produced by increasing $[\text{Ca}^{2+}]_i$ by inhibiting the Na^+ - K^+ pump with strophanthidin^{13,14}. Subsequently increasing $[\text{Ca}^{2+}]_o$ from 2 to 8 mM (Fig. 1B, b) increased resting $[\text{Ca}^{2+}]_i$ and, again, increased both the amplitude and frequency of the oscillations of $[\text{Ca}^{2+}]_i$ as well as producing an aftercontraction. The changes of resting $[\text{Ca}^{2+}]_i$ in Fig. 1 confirm those found with Ca^{2+} -selective microelectrodes^{13–15}.

The above results show that $[\text{Ca}^{2+}]_i$ can oscillate following a twitch. In subsequent experiments we have investigated whether $[\text{Ca}^{2+}]_i$ can oscillate in unstimulated preparations. Because aequorin light is usually undetectable in our preparations at normal resting $[\text{Ca}^{2+}]_i$, in these experiments $[\text{Ca}^{2+}]_i$ was elevated by removing external Na^+ . Figure 2 shows the effects of Na^+ removal (substituted by choline) on tension and light; Na^+ removal produces an increase of both $[\text{Ca}^{2+}]_i$ and tension (Fig. 2A). We have investigated whether this increase of $[\text{Ca}^{2+}]_i$ is accompanied by oscillations of $[\text{Ca}^{2+}]_i$. The problem

with looking for such oscillations is that, even if $[Ca^{2+}]_i$ is steady, the light records will be noisy due to random photon emission by aequorin. This photon noise has a Poisson distribution and the records were therefore analysed to determine whether the variance was greater than that expected from Poisson noise. Measurements of the variance showed that it was 57% greater than that produced by a sample of aequorin producing the same mean light level ($P < 0.01$). The extra component of variance was examined by using a Fourier transform of the light record (Fig. 2B). The aequorin standard gave a flat spectrum as expected for a random source. However, the spectrum obtained from the muscle in Na^+ -free solution shows a sharp peak at ~ 4 Hz, providing direct evidence for a nonrandom oscillation of light and therefore of $[Ca^{2+}]_i$. As measurements during this period showed that the membrane potential did not change by more than 3 mV, these fluctuations of $[Ca^{2+}]_i$ are probably not due to changes in membrane potential. A related observation is

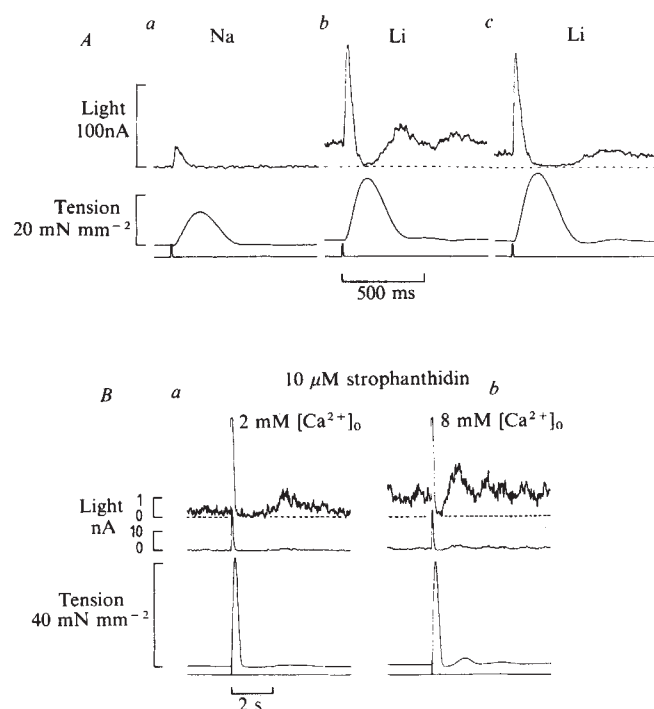


Fig. 1 Effect of increasing the resting $[Ca^{2+}]_i$ on tension and aequorin light in response to stimulation. **A**, All the Na^+ in the superfusing solution was replaced with lithium. Traces show aequorin light (top), tension (middle) and stimulus marker (bottom) averaged ($n = 8$): *a*, in control (Na^+ -containing) solution; *b*, immediately after external Na^+ had been replaced with lithium; *c*, starting 1 min after replacement of external Na^+ with lithium. All solutions were buffered with HEPES. Stimulation rate, 0.33 Hz. **B**, Effects of strophanthidin and $[Ca^{2+}]_0$ on aequorin light and tension. Traces show averaged ($n = 8$) records of light at high gain (top), light at low gain (middle) and tension (bottom). The stimulus marker is shown underneath. *a*, After exposure to 10 μM strophanthidin for 1 h; *b*, 5 min after increasing $[Ca^{2+}]_0$ from 2 to 8 mM in the continued presence of strophanthidin. All solutions were buffered with CO_2/HCO_3^- . Stimulation rate, 0.1 Hz.

Methods: The solution used in the experiments of Figs 1B and 3 contained (in mM): Na^+ 135, K^+ 5, Mg^{2+} 1, Ca^{2+} 2, Cl^- 104, HCO_3^- 20, HPO_4^{2-} 1, acetate 20, glucose 10, insulin 4×10^{-5} and was equilibrated with 95% $O_2/5\%$ CO_2 (pH 7.4). In some experiments in which Na^+ was to be replaced (Figs 1A, 2 and 4) we used HEPES-buffered solutions. In this case the control solution contained (in mM): Na^+ 135; K^+ 5, Mg^{2+} 1, Ca^{2+} 2, Cl^- 141, HEPES 5, glucose 10, insulin 4×10^{-5} and was equilibrated with 100% O_2 (pH 7.4). Na^+ -free solutions containing either lithium, choline or potassium could then be made by substituting the NaCl with the chloride salt of the appropriate cation. No significant differences were noted between experiments carried out in the CO_2/HCO_3^- and the HEPES-buffered solutions. All experiments were done at 30 $^{\circ}C$.

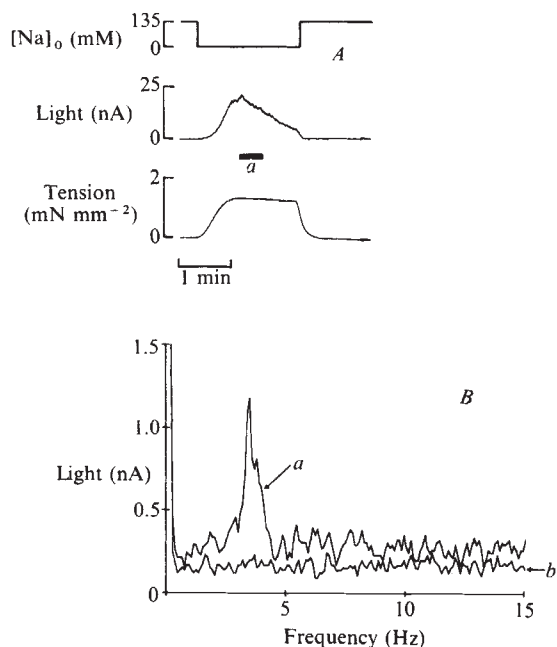


Fig. 2 Effect of Na^+ removal on aequorin light and tension. **A**, Original traces of filtered (1 s time constant) aequorin light (top) and tension (below). The solution protocol is illustrated above the record. The preparation was initially in a Na^+ -containing solution and external Na^+ was replaced by choline for the period indicated. The more rapid rise and fall of the light signal compared with the tension probably reflects the fact that aequorin-injected cells are on the surface of the preparation and therefore less subject to diffusion delays than tension (which is produced by cells across the whole cross-section of the muscle). All solutions were HEPES-buffered. **B**, Fourier amplitude spectra of the aequorin light. Two spectra are superimposed: *a*, obtained in Na^+ -free solution during the period shown in **A**; *b*, obtained from a sample of aequorin and calcium adjusted to give about the same mean light level as in *a*. Spectra were calculated from photomultiplier current signals (bandwidth 0–100 Hz) using a Hewlett Packard 3582A spectrum analyser. The sampling rate was 100 MHz and each spectrum is the average of four 10-s records.

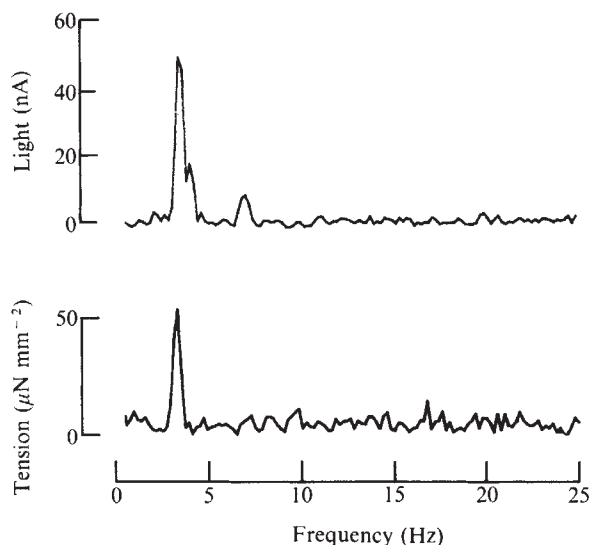
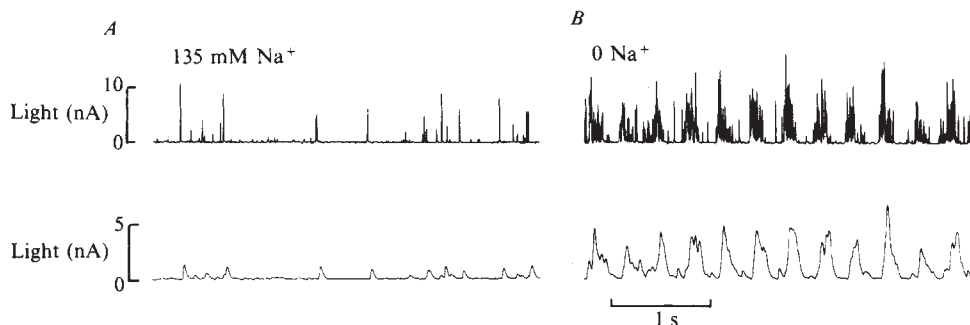


Fig. 3 Fourier amplitude spectra of aequorin light (top) and tension (bottom) during exposure to Na^+ -free solutions. The records were obtained at the peak of a contraction induced by replacing external Na^+ with K^+ . All solutions were buffered with CO_2/HCO_3^- . The spectra were obtained as described in Fig. 2 legend. The mean aequorin light intensity at the peak of the contraction was 1 μA .

Fig. 4 Effects of Na^+ removal on aequorin light emission from a preparation in which less than 10 cells had been injected. In *A* and *B*, the top trace is an aequorin light record obtained at bandwidth 0–300 Hz. The vertical deflections thus correspond to the arrival of individual photons. The lower trace was filtered (bandwidth 0–30 Hz). Panels show records obtained: *A*, in a Na^+ -containing solution (control); *B*, at the peak of a Na^+ -free (replaced by K^+) contraction. All solutions contained strophanthidin (10 μM) and were HEPES-buffered. The calibration procedure at the end of the experiment showed that 5 nA corresponded to a fractional luminescence of 0.0068 and therefore to a $[\text{Ca}^{2+}]_i$ of between 15 and 40 μM (see below).

Methods: The aequorin light signal was calibrated using a method described previously^{1,16}. Briefly, the aequorin in the cells of the preparation was discharged at the end of the experiment by destroying the cell membranes with Triton X-100. Light signals obtained during the experiments could then be normalized to the total aequorin present to give a fractional luminescence. This was converted to $[\text{Ca}^{2+}]_i$ using a calibration curve obtained *in vitro* in conditions designed to mimic the intracellular environment. One complication with the calibration in this experiment is that we do not know how many of the 10 injection attempts were satisfactory and whether the oscillations in $[\text{Ca}^{2+}]_i$ were synchronized in the different injected cells. Two extreme estimates can be made: (1) if 10 cells were injected and the oscillations of $[\text{Ca}^{2+}]_i$ were unsynchronized, the peak $[\text{Ca}^{2+}]_i$ would have been $\sim 40 \mu\text{M}$; (2) if only one cell was injected or the oscillations of all the injected cells were synchronized, the peak $[\text{Ca}^{2+}]_i$ would have been $\sim 15 \mu\text{M}$.



that oscillations with a similar frequency occurred both (1) after replacement of Na^+ by choline (Fig. 2) when the membrane potential was $\sim -70 \text{ mV}$ and (2) after replacement of Na^+ by K^+ (Fig. 3) when the membrane potential was $\sim 0 \text{ mV}$. In five preparations in Na^+ -free solutions, the largest peak of the light spectrum always occurred in the frequency range 3–5 Hz. Figure 3 shows results from a similar experiment in which a Fourier analysis was performed on both light and tension. There is a peak in the tension spectrum at the same frequency as that in the light spectrum. The peak in the tension spectrum was not always so evident, even when there was an obvious peak in the light spectrum. This may be because the compliance of non-contracting cells attenuates the force produced by those which are contracting.

A similar spectral analysis has been performed on tension and membrane current in Purkinje fibres⁵ in which the Na^+ pump had been inhibited to elevate $[\text{Ca}^{2+}]_i$; both spectra showed a peak at $\sim 1 \text{ Hz}$. The authors noted that manoeuvres which were assumed to decrease $[\text{Ca}^{2+}]_i$ decreased not only the magnitude but also the frequency of the peak. Similarly, Fig. 1 shows that the frequency of the oscillations of $[\text{Ca}^{2+}]_i$ decreases as the mean $[\text{Ca}^{2+}]_i$ falls. As exposure to Na^+ -free solutions elevates $[\text{Ca}^{2+}]_i$, more than does Na^+ pump inhibition (Fig. 1), the higher frequency of fluctuations observed in the present work is not unexpected.

These oscillations of $[\text{Ca}^{2+}]_i$ will have important effects on the interpretation of Ca^{2+} and tension measurements from resting preparations. Since, at least at the higher levels of $[\text{Ca}^{2+}]_i$ investigated here, aequorin light emission is proportional to $[\text{Ca}^{2+}]_i^{2.5}$ (ref. 16), oscillations of $[\text{Ca}^{2+}]_i$ will produce a mean light greater than that produced by a steady $[\text{Ca}^{2+}]_i$ with the same mean value. Conversely, Ca^{2+} -sensitive electrodes, which have a logarithmic dependence on $[\text{Ca}^{2+}]_i$, will underestimate the mean $[\text{Ca}^{2+}]_i$. Furthermore, if the fluctuations are not synchronized between different cells, the tension will be less than the mean isometric tension of each cell. This complication, together with that due to the nonlinear Ca^{2+} -aequorin relationship, can explain the observation that, during a Na^+ -free contraction, a given amount of aequorin light is accompanied by much less tension than during the twitch¹⁷ (Fig. 1A). Presumably during the twitch $[\text{Ca}^{2+}]_i$ in all cells is synchronized.

A disadvantage of the experiments shown in Figs 2 and 3 is that many cells were injected. Therefore, if the oscillations of $[\text{Ca}^{2+}]_i$ in different cells are not synchronized, macroscopic oscillations may not be evident and will only be detected by spectral analysis. One way to overcome this problem is to inject only a few cells. We attempted to inject only 10 cells within a

small region of the muscle (see Fig. 4); the number of successful injections is likely to have been less than 10. The results show that, in Na^+ -free solution, the photon emission occurred in characteristic bursts. The filtered record shows that $[\text{Ca}^{2+}]_i$ oscillated with a mean frequency of 3 Hz. We estimate (see Fig. 4 legend) that the peak level of $[\text{Ca}^{2+}]_i$ reached during these oscillations was at least 15 μM ; the minimum value was undetectable.

These oscillations of $[\text{Ca}^{2+}]_i$ may be due to the oscillatory release of Ca^{2+} from the sarcoplasmic reticulum, perhaps via a Ca^{2+} -induced Ca^{2+} release similar to that proposed for skinned cardiac cells⁷. The periodicity of the oscillations in the present study is within the range observed in skinned cardiac cells. The involvement of the sarcoplasmic reticulum is also supported by the observation that caffeine abolishes the spontaneous cellular movements¹⁸ and the oscillatory aftercontraction and transient inward current^{19,20}. Similarly, we have found (results not shown) that caffeine (10 mM) abolishes the fluctuations of $[\text{Ca}^{2+}]_i$ in Na^+ -free solutions. These results suggest that the sarcoplasmic reticulum can contribute to the control of $[\text{Ca}^{2+}]_i$, even in quiescent muscles and that caution is required in ascribing the control of resting $[\text{Ca}^{2+}]_i$ to surface membrane mechanisms.

The fluctuations of $[\text{Ca}^{2+}]_i$ reported here presumably underlie the fluctuations of tension and current reported previously. It is probable that the undershoot and subsequent oscillations of $[\text{Ca}^{2+}]_i$ which follow the twitch (Fig. 1) are produced by the synchronization of these, otherwise random, oscillations (see also, ref. 5). In the present experiments it was necessary to elevate $[\text{Ca}^{2+}]_i$ to resolve these fluctuations of $[\text{Ca}^{2+}]_i$, as the aequorin signal is undetectable at normal $[\text{Ca}^{2+}]_i$. Spontaneous cellular movements have, however, been reported⁶ in cardiac muscle in normal conditions. It will be interesting, therefore, to characterize oscillations of $[\text{Ca}^{2+}]_i$ in more physiological conditions.

We thank P. Gray and O. Lippold for their help with the Fourier analyses, and M. Cannell and E. Lakatta for valuable discussion. The aequorin used in this study was prepared in the laboratory of Dr J. R. Blinks under NIH grant HL 12186. This work was supported by grants from the MRC, the British Heart Foundation and the Royal Society.

Received 4 March; accepted 6 July 1983.

- Allen, D. G. & Blinks, J. R. *Nature* **273**, 509–513 (1978).
- Glitsch, H. G. & Pott, L. *Pflügers Arch. ges. Physiol.* **358**, 11–25 (1975).
- Kass, R. S., Lederer, W. J., Tsien, R. W. & Weingart, R. J. *J. Physiol., Lond.* **281**, 187–208 (1975).
- Eisner, D. A. & Lederer, W. J. *J. Physiol., Lond.* **294**, 255–277 (1979).
- Kass, R. S. & Tsien, R. W. *Biophys. J.* **38**, 259–269 (1982).

6. Lakatta, E. G. & Lappe, D. L. *J. Physiol., Lond.* **315**, 369–394 (1981).
7. Fabiato, A. & Fabiato, F. *J. Physiol., Lond.* **249**, 469–495 (1975).
8. Reiter, M. *Naunyn-Schmiedeberg's Archs Pharmacol.* **242**, 497–507 (1962).
9. Colquhoun, D., Neher, E., Reuter, H. & Stevens, C. F. *Nature* **294**, 752–754 (1981).
10. Allen, D. G. & Orchard, C. H. *J. Physiol., Lond.* **335**, 555–567 (1983).
11. Reuter, H. & Seitz, N. *J. Physiol., Lond.* **195**, 451–470 (1968).
- Blinks, J. R., Wier, W. G., Morgan, J. P. & Hess, P. in *Advances in Pharmacology and Therapeutics II* Vol. 3 (eds Yoshida, H., Hagihara, Y. & Ebashi, S.) (Pergamon, Oxford, 1982).
13. Bers, D. M. & Ellis, D. *Pflügers Arch. ges. Physiol.* **393**, 171–178 (1982).
14. Sheu, S.-S. & Fozzard, H. A. *J. gen. Physiol.* **80**, 325–351 (1982).
15. Lee, C. O., Uhm, D. Y. & Dresdner, K. *Science*, **209**, 699–701 (1980).
16. Allen, D. G. & Blinks, J. R. in *Detection and Measurement of Free Ca²⁺ in Cells* (eds Ashley, C. C. & Campbell, A. K.) 159–174 (Elsevier, Amsterdam, 1979).
17. Allen, D. G., Eisner, D. A., Lab, M. J. & Orchard, C. H. *J. Physiol., Lond.* (in the press).
18. Stern, M. D., Kort, A. A., Bhatnagar, G. M. & Lakatta, E. G. *Biophys. J.* **33**, 284a (1981).
19. Eisner, D. A. & Lederer, W. J. *J. Physiol., Lond.* **322**, 48P–49P (1982).
20. Karagueuzian, H. S. & Katzung, B. G. *J. Physiol., Lond.* **327**, 255–271 (1982).

Ligand-induced rapid redistribution of lysosomes is temporally distinct from endosome translocation

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When many ligands bind to cell-surface receptors, ligand-receptor complexes are internalized via clathrin coated pits by a process called receptor-mediated endocytosis^{1,2}. The cytoplasmic fate of ligands internalized within endocytic vesicles or endosomes is variable. For example, maternal immunoglobulins are transported through the cytoplasm of neonatal intestinal epithelial cells and are exocytosed at the basolateral surface³. However, other ligands are degraded as a result of their delivery to the lysosomal compartment of cells^{2,4–6}. Although the translocation of endosomes to the Golgi region in the cell centre seems to be a general phenomenon presumably coupled to ligand degradation by lysosomes^{7,8} and endosomes and lysosomes undergo saltatory movements within the cytoplasm^{8,9}, the spatial control of interaction between the two structures is not understood. To address this problem we have begun to examine the spatial and temporal intracellular distribution of endosomes and lysosomes. Utilizing a new fluorescent microscopic approach¹⁰, we have now been able simultaneously to visualize endosome and lysosome populations in living cells. Our results suggest that a specific relocation of lysosomes is rapidly induced upon binding of different types of ligands to the cell surface; this migration of lysosomes to the Golgi region of the cell precedes the translocation of endosomes into the same area.

Figure 1 illustrates the time dependent reorganization of lysosomes and endosomes in 5-day-old cultures of ovarian granulosa cells after addition of concanavalin A (Con A), a mitogenic lectin taken up by receptor mediated endocytosis¹¹. In living cells, >70% of the cells exhibit dispersed lysosomes, visualized as phase dense particles (Fig. 1A) which stain with the specific lysosomal marker acridine orange^{12–14} (Fig. 1B). Lysosomes remain dispersed after exposing cells for 10 min to pyrene-Con A (P-Con A), which labels a diffusely distributed population of surface receptors (Fig. 1C). By 20 min of labelling with P-Con A, ligand receptor complexes are internalized within endosomes which are randomly distributed throughout the cytoplasm (Fig. 1D). During the 20-min time interval in which endosomes form, acridine orange staining of the same cells indicates that lysosomes have undergone a striking relocation to the perinuclear region of the cell. Additionally, cells were stained with acridine orange 30 min after exposure to P-Con A. In these cells which contain a dispersed population of endosomes, we found that acridine orange staining was confined to perinuclear lysosomes (data not shown). This finding is consistent with the lysosome-specific nature of acridine orange staining¹⁴ which is known to be affected by factors other

Table 1 Ligand-induced redistribution of lysosomes in cultured ovarian granulosa cells

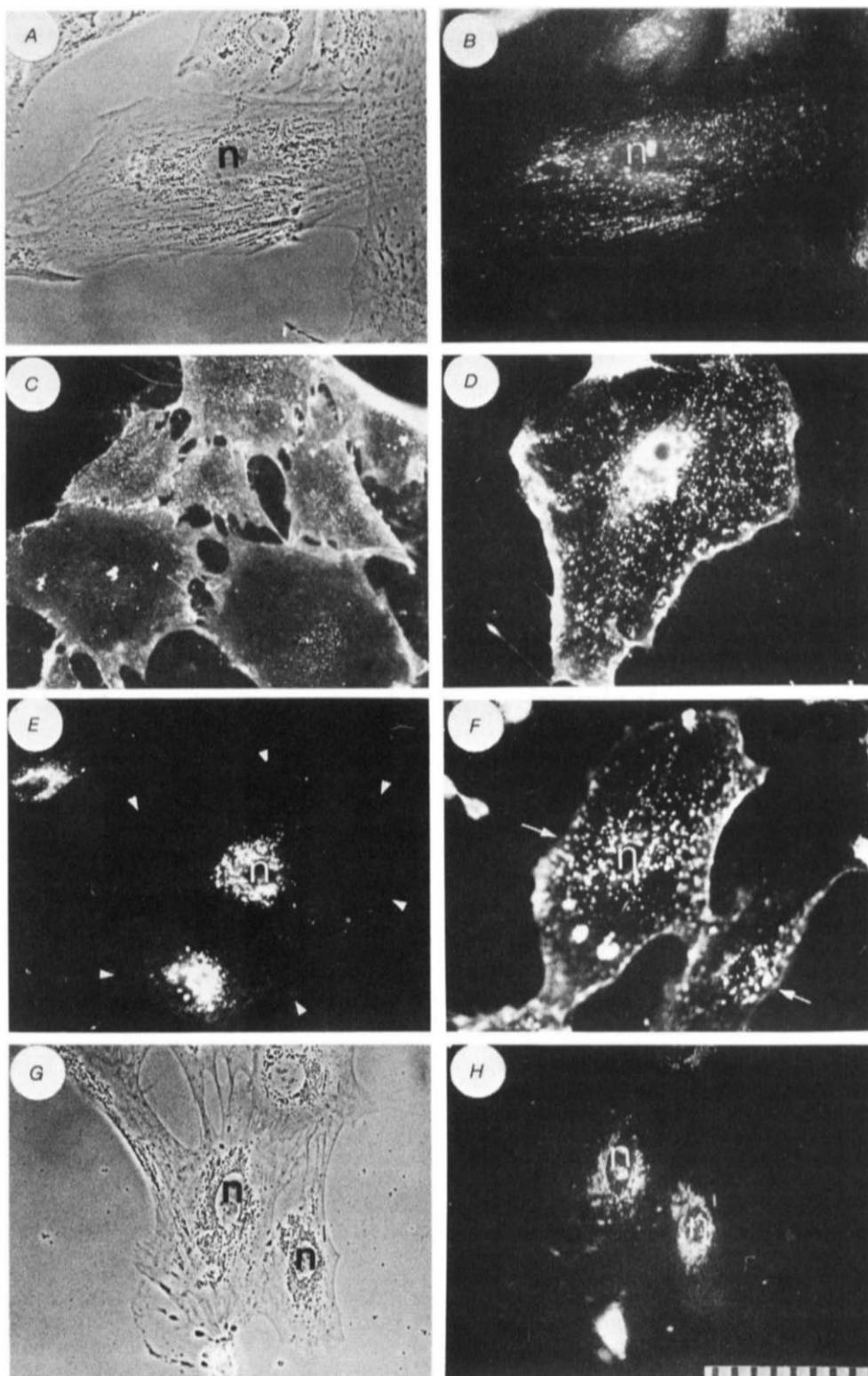
	Time (min)					
	30			60		
	Lysosome distribution					
	D(%)	A(%)	χ^2	D(%)	A(%)	χ^2
Ligands						
None (control)	71	29	—	71	29	—
EBSSH	69	31	0.2	75	25	0.8
Con A	34	66	48.3	43	57	31.8
Succinyl-Con A	38	62	37.4	48	52	18.2
Wheat germ agglutinin	53	47	13.9	57	43	7.8
<i>Ricin communis</i>	56	44	9.7	56	44	9.7
Epidermal growth factor	41	59	38.7	40	60	39.0
17 β -oestradiol	46	54	25.7	37	63	47.5
FSH	38	62	42.1	—	—	ND
Cyclic AMP	38	62	37.8	—	—	ND
Human chorionic gonadotropin	66	34	0.2	—	—	ND
Chondroitin sulphate	47	53	22.5	48	52	18.7
Dermatan sulphate	47	53	22.8	44	56	28.7
Heparin sulphate	45	55	27.7	50	50	8.0
Hyaluronic acid	42	58	33.1	34	66	42.5
Insulin	68	32	0.6	73	27	0.1
Transferrin	58	42	8.3	63	37	2.9
Medium readdition	65	35	1.3	52	48	16.4

150–200 cells were counted on coverslips of granulosa cells cultured from oestrogen primed immature female rats as previously described²⁷. The distribution of lysosomes was scored as dispersed (D), (as in Fig. 1A and B), or aggregated (A), (as in Fig. 1E, G and H). Cells were grown in complete McCoy's 5A medium containing 10% newborn calf serum for 5 days. The medium was removed, cells were rinsed twice in EBSSH and exposed to various ligands in EBSSH for 10 min at 37 °C. Cells were then washed twice with EBSSH (without ligand) and incubated for a further 30 to 60 min in EBSSH at 37 °C at which time lysosomal distribution was determined as described in the legend to Fig. 1. The following concentrations of ligands were used: Con A, 100 $\mu\text{g ml}^{-1}$; succinyl Con A, 100 $\mu\text{g ml}^{-1}$; wheat germ agglutinin, 100 $\mu\text{g ml}^{-1}$; Ricin communis, 100 $\mu\text{g ml}^{-1}$; 17 β -oestradiol, 10⁻⁷ M; FSH, 10 $\mu\text{g ml}^{-1}$; cyclic AMP, 0.5 mM; human chorionic gonadotropin, 100 ng ml⁻¹; chondroitin sulphate, 100 $\mu\text{g ml}^{-1}$; dermatan sulphate, 100 $\mu\text{g ml}^{-1}$; heparin sulphate, 100 $\mu\text{g ml}^{-1}$; hyaluronic acid, 100 $\mu\text{g ml}^{-1}$; epidermal growth factor, 10 ng ml⁻¹; insulin, 4 $\mu\text{g ml}^{-1}$; and human transferrin, 5 $\mu\text{g ml}^{-1}$. Control cultures were continually incubated in complete medium; EBSSH cultures were incubated for the specified times in EBSSH without additional ligands; for medium readdition, cells were incubated for 60 min in EBSSH, complete medium was then added and cells were scored as above. χ^2 analysis of the raw data was performed on a 2 $\times$ 2 contingency table with one degree of freedom as described in ref. 26. For the change in lysosome distribution to be significant at the 0.1% level the χ^2 value +10.83. ND, not determined; cells undergo rounding response within 60 min of exposure to FSH or cyclic AMP.

than pH alone¹⁵. As shown in Fig. 1E, virtually all of the lysosomes have relocated to the cell centre around the nucleus. Endosomes containing P-Con A gradually migrate to the cell centre over a 60-min time span (Fig. 1F) whereas the central accumulation of lysosomes previously induced by P-Con A remains unchanged (Fig. 1G, H). At these time points, overlap between endosomes and lysosomes is apparent in the perinuclear region of the cell where electron microscopic studies have shown that endosomes fuse with lysosome-like structures¹¹. Note that no appreciable change in cellular shape occurs during this process. These results demonstrate that ligand binding causes lysosomes to migrate to the cell centre before the directed translocation of endosomes to the same region of these cells.

To determine whether the effect of Con A on lysosome redistribution is unique to this lectin or represents a general response to physiological ligands, we examined the effects of a number of ligands on this process. The results of these experiments are summarized in Table 1. Most of the agents tested

Fig. 1 Corresponding phase (A, G), acridine orange (B, E, H) and pyrene-Con A (P-Con A, C, D, F) fluorescence micrographs of 5-day rat ovarian granulosa cell cultures. After a 10-min exposure to $100 \mu\text{g ml}^{-1}$ P-Con A at 37°C , many of the phase dense particles observed in living cells (A) correspond to acridine orange positive structures which are distributed throughout the cytoplasm (B). P-Con A binds to a diffusely distributed population of cell-surface receptors (C). By 30 min after P-Con A addition, ligand is internalized¹¹ in endosomes that are distributed over the nucleus and throughout the cytoplasm (D). At this same time, acridine orange stained structures have relocated to the cell centre (E). At 60 min after P-Con A labelling, endosomes continue to migrate to the cell centre (F) whereas the central predisposition of phase dense (G) acridine orange staining structures is maintained (H). Cultures were established as previously described²⁷ and P-Con A was prepared as described in ref. 28. Cells grown on glass coverslips were labelled for 10 min at 37°C with $100 \mu\text{g ml}^{-1}$ P-Con A in phosphate buffer saline (PBS). After three rinses in PBS, cells were exposed to $10 \mu\text{g ml}^{-1}$ acridine orange in EBSSH for 1 min at room temperature. The coverslip was washed twice in EBSSH and mounted in a brass chamber designed for continual monitoring of fluorescence under a Zeiss photomicroscope III equipped with epifluorescence illumination. Photographs were recorded on Kodak Tri-X film push processed in Acufine developer at an ASA of 1,600. The epiillumination system was adapted to permit observation of P-Con A or acridine orange fluorophores either individually or simultaneously; both fluorophores excite at 380 nm whereas the emission maximum for P-Con A is 460 nm and 600 nm for acridine orange²⁸. The insertion of a 500 nm cutoff filter allows for observation of the pyrene independent of acridine orange fluorescence; a 365 nm interference filter is used with a 395 nm dichroic reflector for sample excitation. n, Nucleus; arrowheads, cell margin. All photographs are of the same magnification and the scale bar in H equals 10 μm divisions.



induced some degree of lysosomal aggregation within 30 min after exposure to a specific ligand. The reagents tested included various lectins, specific hormones which modulate growth and differentiation of rat granulosa cells^{16,17}, certain extracellular matrix molecules which influence granulosa cell function¹⁸, and finally, several factors required for the maintenance of granulosa cells in serum-free culture conditions¹⁹. Living cells were scored as exhibiting dispersed (Fig. 1B) or aggregated (Fig. 1E) lysosome patterns after staining with acridine orange.

Negligible changes in lysosome distribution were observed in untreated (control) cultures, cultures exposed to balanced salts alone (EBSSH), or cultures to which fresh medium was

added after a 60-min incubation in EBSSH. Of the lectins tested, all were effective at inducing lysosome aggregation and their order of potency, at equivalent concentrations, was found to be Con A > succinyl-Con A > wheat germ agglutinin > *Ricin communis*. Thus, lectins which vary in their valency and sugar binding characteristics were effective in eliciting lysosomal redistribution. 17β -oestradiol, follicle stimulating hormone (FSH), cyclic AMP and epidermal growth factor (EGF) also elicited a change in lysosome distribution when added to these cells. Since cyclic AMP produced a near maximal response this may indicate that effector molecules directly induce lysosome movement independent of receptor occupation. Human

chorionic gonadotropin (hCG), which causes granulosa cell differentiation, was without effect. These results are consistent with the fact that the granulosa cells used in this study have been shown to possess receptors for 17β -oestradiol, FSH, and EGF, but at this stage of differentiation lack receptors for hCG^{16,17}. The various glycosaminoglycans tested were also able to cause lysosome redistribution. Readdition of serum containing medium, or exposure to either insulin or transferrin alone resulted in only a partial redistribution of lysosomes which was not significantly different from controls. The presence of insulin and transferrin in the medium used to culture granulosa cells may have led to the down-regulation of receptors for these factors and probably accounts for the partial response observed.

Two important questions are posed by our findings. The first relates to the mechanism cells utilize to orchestrate and direct the movement of two distinct classes of intracellular organelles to specific sites within the cytoplasm. Second, how general is this response and what is its biological significance? The mechanism of regulation of rapid lysosome redistribution is not known but the gradual accumulation of endosomes in the Golgi region of cells seems to be a general phenomenon since this behaviour has been observed for a number of cell types undergoing receptor mediated endocytosis of a variety of ligands^{2,7,8}. The movement of endosomes is presumably responsible for the delivery of cell-surface derived ligand-receptor complexes to specific regions of the cell; these excursions cover distances in excess of 10 μ m in cultured cells and may be related to the biological action of the ligand by enhancing the interaction of endosomes with other cytoplasmic constituents. For example, low density lipoprotein and epidermal growth factor, two ligands known to be internalized by receptor mediated endocytosis, are delivered to lysosomes where degradation occurs^{5,6,20}. Although Con A has been a suitable marker for endosomes in this system, previous studies have demonstrated that Con A can inhibit lysosome-phagosome fusion in macrophages²¹. In view of this potential side effect of Con A, it will be necessary to examine endosome-lysosome interactions with fluorescently conjugated physiological ligands.

Our results also demonstrate that lysosomes undergo a migration spatially similar to that of endosomes, but that lysosomes migrate before the endosomes. The aggregation of lysosomes to the cell centre may provide a mechanism for non-randomizing the cytoplasmic location of this organelle so that upon endosome accumulation in the cell centre, the probability of interactions between these two cytoplasmic compartments will be increased. Aortic smooth muscle cells, growth arrested by serum deprivation, undergo rapid lysosome aggregation in response to platelet derived growth factor or serum addition (B.H. and J. J. Castellot, unpublished). The rapid migration of lysosomes to the cell centre has been noted in other hormone-responsive cell types. For example, luteinizing hormone causes a rapid aggregation of lysosomes around the germinal vesicle of rat oocytes during meiotic maturation²² and oestrogens elicit a similar response in uterine endometrial cells²³. Thus, lysosome aggregation and perhaps the aggregation of other organelles²³, may represent a general response in target cells on exposure to physiological ligands.

The regulation of organelle movement in cells is under study. The cytoskeleton has been implicated in the regulation of various types of cytoplasmic organelle motion^{9,24} and recent evidence suggests that the cytoskeleton may be involved in controlling lysosome and endosome translocation in cells. We have described alterations in the organization of microtubules and intermediate filaments that occur coincident with endosome movement to the cell centre¹¹. Moreover, pharmacologically induced alterations in microtubule structure inhibit endosome translocation¹¹ and impair the degradation of EGF²⁵, a ligand shown here to cause lysosomal aggregation. Thus, it seems likely that formed structural elements of the cytoplasm may play a part in regulating interactions between intracellular membrane compartments.

The use of P-Con A and acridine orange double staining in living cells has allowed us to distinguish between the movements of two distinct organelles through the cytoplasm. The importance of the observed endosome and lysosome reorganization with respect to the biological effects of these ligands has yet to be determined; however, this process may represent a basic cellular response designated for the effective catabolism of ligands which enter cells through a receptor mediated process. Future studies should be aimed at understanding the mechanisms responsible for organelle movement and interactions.

The work was supported by NSF grants PCM 78-16158 and PCM 79-27781 and a Milton Fund grant to D.F.A. We thank Ms Liz Dreesen and Ms Kay Cosgrove for help in preparation of the manuscript, the NICHD for hCG, and the National Pituitary Agency, NIAMDD for rat FSH.

Received 6 March; accepted 22 June 1983.

- Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. *Nature* **279**, 679-685 (1979).
- Steinman, R. M., Mellman, I. S., Muller, W. A. & Cohn, Z. A. *J. Cell Biol.* **96**, 1-28 (1983).
- Abrahamson, D. & Rodewald, R. *J. Cell Biol.* **91**, 270-280 (1981).
- Ascoli, M. & Puett, D. *J. Biol. Chem.* **253**, 4892-4899 (1978).
- Greenburg, P. L. & Rozengurt, E. *Exp. Cell Res.* **142**, 111-117 (1982).
- Anderson, R. G. W., Brown, M. S. & Goldstein, J. L. *Cell* **10**, 351-364 (1977).
- Maxfield, F. R., Schlesinger, J., Schechter, Y., Pastan, I. H. & Willingham, M. C. *Cell* **14**, 805-810 (1978).
- Pastan, I. H. & Willingham, M. C. *Science* **214**, 504-509 (1981).
- Freed, J. J. & Lebowitz, M. M. *J. Cell Biol.* **45**, 334-354 (1970).
- Herman, B. & Albertini, D. F. *J. Cell Biol.* **95**, 440a (1982).
- Herman, B. & Albertini, D. F. *Cell Motility* **2**, 583-597 (1982).
- Allison, A. C. & Young, M. R. *Life Sci.* **3**, 1407-1414 (1964).
- Zelenin, A. V. *Nature* **212**, 425-426 (1966).
- Robbins, E., Marcus, P. I. & Gonatas, N. K. *J. Cell Biol.* **21**, 49-62 (1964).
- Robbins, E. & Marcus, P. I. *J. Cell Biol.* **18**, 237-250 (1963).
- Richards, J. S. *Physiol. Rev.* **60**, 51-89 (1980).
- Mondschein, J. S. & Schomberg, D. W. *Science* **211**, 1179-1180 (1981).
- Eppig, J. J. *Endocr.* **108**, 1992-1994 (1981).
- Orly, J., Sato, G. & Erickson, G. F. *Cell* **20**, 817-827 (1980).
- Carpenter, G. & Cohen, S. *J. Cell Biol.* **71**, 159-171 (1976).
- Edelson, P. J. & Cohn, Z. A. *J. exp. Med.* **140**, 1364-1386 (1974).
- Ezzell, R. M. & Szego, C. M. *J. Cell Biol.* **82**, 264-277 (1979).
- Szego, C. M. & Seeler, B. J. *J. Endocr.* **56**, 347-360 (1973).
- Wang, E. & Goldman, R. D. *J. Cell Biol.* **79**, 708-726 (1978).
- Brown, K. D., Friedkin, M. & Rozengurt, E. *Proc. natn. Acad. Sci. U.S.A.* **77**, 480-484 (1980).
- Bailey, N. T. J. in *Statistical Methods in Biology*, 2nd edn, 52 (Wiley, New York, 1981).
- Albertini, D. F. & Kravitz, N. K. *J. Biol. Chem.* **256**, 2484-2492 (1981).
- Herman, B. & Fernandez, S. M. *Biochemistry* **21**, 3271-3274 (1982).

56K fibroblast protein not specific for Duchenne muscular dystrophy but for skin biopsy site

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Recently, we reported the detection by two-dimensional gel electrophoresis of a 56,000 (56K)-molecular weight polypeptide that was present in cultured skin fibroblasts from normal males but was not detectable in fibroblasts from Duchenne muscular dystrophy (DMD) patients¹. Since then we have established that the 56K polypeptide is not present in skin fibroblasts from normal females, DMD carrier females, or in normal male fibroblasts obtained from sources other than the Repository for Mutant Human Cell Strains, Montreal. Further inquiry has led to the discovery that all the normal male fibroblast cultures obtained from the Montreal repository had been established from preputial skin. Fibroblast cultures from DMD patients, DMD carriers, as well as other normal individuals, had been derived from non-genital skin biopsies. Thus, the absence of the 56K polypeptide is not specific for DMD, but rather is related to the site of skin biopsy.

Generally, little significance has been attached to the site of the skin biopsy used to establish fibroblast cultures. Yet the potential dangers of such an oversight, when studying mutant cell strains, have been pointed out earlier². The biopsy site is

often not given in cell repository catalogues or on the specification sheets that accompany cell cultures obtained from the repositories. These omissions have led to the unintentional comparison of normal genital skin fibroblasts with non-genital skin fibroblasts from patients with genetic diseases (see for example refs 1, 3–5). Therefore, our earlier interpretation¹ that the 56K polypeptide might be specific for DMD was incorrect.

What, then, is the significance of the qualitative difference in the protein maps of genital and non-genital fibroblasts? As genital skin fibroblasts are androgen target cells⁶, the 56K polypeptide may be an androgen receptor or an androgen-dependent protein. It will be of interest to test the possible involvement of this protein in cells from patients with androgen resistance syndromes, a heterogeneous group of disorders of genotypical males with female phenotypes^{7,8}.

It is possible that there are additional qualitative and quantitative differences in protein composition between fibroblasts from genital and non-genital skin sites. However, complete two-dimensional gel maps cannot be reliably compared by visual inspection of separate stained gels or single-label autoradiographs. We intend to make such comparisons in the future using double-label autoradiographic techniques^{9,10}.

We thank A. Vust and R. Torres for technical assistance. This work was supported by the Muscular Dystrophy Association of Canada, the Manitoba Heart Foundation and the MRC of Canada.

Received 22 April; accepted 14 June 1983.

1. Rosenmann, E. *et al.* *Nature* **298**, 563–565 (1982).
2. Kaufman, M. & Pinsky, L. *Lancet* **ii**, 1202–1203 (1973).
3. Thompson, R. G. *et al.* *J. neurol. Sci.* **57**, 41–54 (1982).
4. Gelman, B. B. *et al.* *J. clin. Invest.* **65**, 1398–1406 (1980).
5. Rounds, P. S. *et al.* *Biochem. biophys. Res. Commun.* **97**, 1384–1390 (1980).
6. Brown, T. R. & Migeon, C. J. *Molec. cell. Biochem.* **36**, 3–22 (1981).
7. Amrhein, J. A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 891–894 (1976).
8. Migeon, B. R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **78**, 6339–6343 (1981).
9. Walton, K. E., Styer, D. & Gruenstein, E. *J. biol. Chem.* **254**, 7951–7960 (1980).
10. McConkey, E. H. *Analyt. Biochem.* **96**, 39–44 (1979).

Postnatal development of the adult pattern of motor axon distribution in rat muscle

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Establishment of the adult pattern of connections between motoneurons and their muscles is known to be achieved in stages. There is an initial guidance mechanism which in higher vertebrates directs the axons with considerable accuracy to their appropriate muscles¹. This is followed by a stage when a large proportion of neurones die². Then, after birth, the final modifications are brought about by elimination of redundant motoneuronal branches^{3–5}. We present here evidence that branch elimination does not occur at random from within a motoneurone's initial intramuscular distribution, but in such a way as to sharpen the segmento-topic relationship within a muscle that motoneuronal death had earlier provided⁶.

Experiments were carried out on the sheet-like gluteus muscle of neonatal rats aged 0–1 days and 13–17 days. The muscle (Fig. 1) varies between 12 and 25 fibres thick and is innervated by about 20 motor axons. It arises medially from the iliac crest and inserts into the femur and fascia of the thigh, the fibres running more or less parallel to one another. The rats were anaesthetized with ether and then decapitated. The left gluteus muscle, together with the inferior gluteal nerve, the sciatic nerve and the appropriate spinal roots (L3–L6) still attached to the spinal cord were then dissected under oxygenated mammalian Ringer's solution. The gluteus muscle was

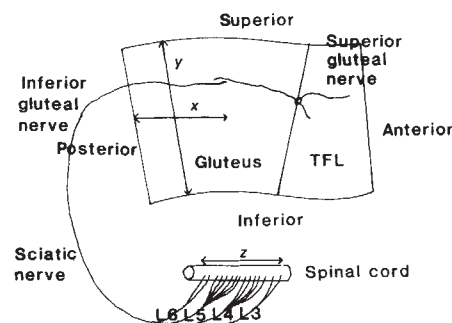


Fig. 1 Diagram of experimental preparation. The left gluteus maximus is viewed from its inner surface. The dimensions x , y and z were on average 2.5, 4.3 and 2.5 mm in 0–1-day-old rats and 4.1, 7.6 and 3.6 mm in 13–17-day-old rats. Only the portion of the muscle innervated by the inferior gluteal nerve was studied, the superior nerve being cut. TFL, tensor fascia latae muscle.

pinned out flat in a sylgard-lined dish at room temperature ($\sim 20^\circ\text{C}$) and the ventral roots were stimulated at their point of exit from the spinal cord by a 50- μm stainless steel cathode. The all-or-none contractions resulting from stimulation with threshold currents were observed through a dissecting microscope. Because of the muscle's thin roughly rectangular shape, such single motor unit contractions can be localized to a discrete region of the muscle⁷. For five positions of the stimulating electrode on the root exit zone in each preparation, the site of contraction of the lowest threshold motor unit was measured with a thin strip of millimetre graph paper pinned across the muscle at about the middle of the fibres.

The results are shown in Fig. 2. In rats older than 13 days (by which time nearly all the redundant branches giving rise to polyneuronal innervation have gone⁸) the width of the muscle occupied by each motor unit averages $14.5 \pm 8.3\%$ ($\pm \text{s.d.}$, $n = 33$) of the territory innervated by the whole inferior gluteal nerve. There is also a clear relationship between the rostro-caudal level of root stimulation and the position in the muscle where the contraction occurs. In the 0–1-day old muscles each unit caused contraction of a band which on average represented $40.4 \pm 16.8\%$ ($n = 33$) of the muscle. The position of the bands was, as in the older rats, significantly correlated with the point of origin of the motor axon from the spinal cord, but the slope of this relationship was significantly less and the degree of correlation weaker.

The methods both of mapping the motor unit position and of activating motor axons from a particular rostro-caudal position are subject to error. In the muscle, the motor unit limits might be overestimated by including muscle fibres whose movements were only due to their being pulled by nearby contracting fibres, and underestimated by our failure to detect movement of fibres contracting beneath the surface ones. However, similar results were obtained in the intercostal muscles of newborn rats, which are similarly two-dimensional, on comparing single motor unit distributions determined by visual inspection, as here, and by means of mapping with microelectrodes⁷. The position of the stimulating electrode on the root was measured to the nearest 0.1 mm. However, as contractions of particular low threshold units could sometimes be obtained from positions on the root differing by up to 0.3 mm, the accuracy of root outflow mapping was only of the same order as that of the motor units within the muscle.

Despite these apparent inaccuracies, it is clear that reduction in motor unit size as polyneuronal innervation is eliminated involves a dramatic decrease in the lateral spread of each motor unit within the muscle. This rules out a random loss of branches. There are two possible alternatives. There is either a symmetrical loss of those branches lying at the two edges of a motor unit's terminal distribution, or a specific loss of those branches which are least appropriately placed on a positional gradient

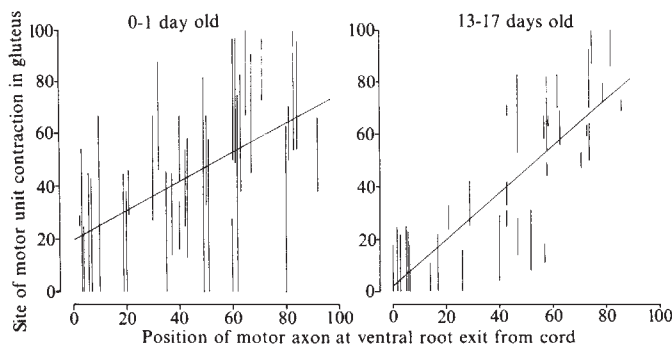


Fig. 2 Correlations between the site of contraction of motor units in a rat gluteus muscle (ordinate) and the rostral-caudal position of the motor axon causing the contraction (abscissa). Positions in the muscle and root have been normalized. In the muscle, 0 represents the posterior margin of the muscle and 100 the most anterior portion of the gluteus made to contract by stimulating the whole of the inferior gluteal nerve. In the root, 0 represents the rostral end of L6 and 100 the rostral end of L3. Nearly all axons to the inferior gluteal nerve emerge in L4 and L5, which are usually the largest lumbar roots. On the left are data from seven rats aged 0–1 days (33 motor units), while data on the right are from seven rats aged between 13 and 17 days (33 motor units). Regression lines calculated using the midpoint of each unit contraction site: for 0–1-day-old rats, slope = 0.54, $r = 0.66$, $P < 0.001$; for 13–17-day-old rats, slope = 0.89, $r = 0.83$, $P < 0.001$. The difference between slopes for the two groups of rats is significant at the 5% level ($F = 4.88$ with 1 and 62 d.f.).

within the muscle. A simple symmetrical pruning of the marginal nerve branches would leave the central point of each motor unit unaltered and thus neither the slope nor the degree of correlation between the root position and muscle position would change. Both the slope and the degree of correlation increase as the muscle innervation matures, thus supporting the view that synapse elimination removes nerve branches which are least well positionally matched with muscle fibres^{9,10}.

Other recent work¹¹ has provided evidence that muscle fibres originating from different segmental levels possess a label indicating their place of origin, which can be detected even by cholinergic nerves which are not motoneurons. A simple hypothesis¹¹ would be that all tissues originating from a particular level during development would acquire a common positional label. In the case of limb muscles this label could be carried from the somites into the limb as the muscle cell precursors migrated^{12,13}. There the labels could subsequently be detected by the motor axons. In most muscles, the segmento-topic organization which can be so easily seen in broad flat muscles like the gluteus and the diaphragm¹⁴ is likely to be harder to detect experimentally, but doubtless the motor axons can do so just as readily as they apparently do in the gluteus. Failure to form adequate synapses because of positional mismatch could be partly responsible for cell death at early stages of neuromuscular development⁶ and contribute to branch withdrawal after birth.

This work was supported by the MRC.

Received 24 May; accepted 6 July 1983.

1. Lance-Jones, C. & Landmesser, L. *Proc. R. Soc. B* **214**, 19–52 (1981).
2. Hamburger, V. & Oppenheim, R. W. *Neurosci. Commentaries* **1**, 39–55 (1982).
3. Redfern, P. A. *J. Physiol., Lond.* **209**, 701–709 (1970).
4. Bennett, M. R. & Pettigrew, A. J. *J. Physiol., Lond.* **241**, 515–545 (1974).
5. Brown, M. C., Jansen, J. K. S. & Van Essen, D. C. *J. Physiol., Lond.* **261**, 387–422 (1976).
6. Bennett, M. R. & Lavidis, N. A. *Dev. Brain Res.* **2**, 448–452 (1982).
7. Dennis, M. J., Ziskind-Conhaim, L. & Harris, A. J. *Dev. Biol.* **81**, 266–279 (1981).
8. Brown, M. C., Hopkins, W. G. & Keynes, R. J. *J. Physiol., Lond.* **329**, 439–450 (1981).
9. Brown, M. C. & Booth, C. M. *Brain Res.* (in the press).
10. Dennis, M. J. & Harris, A. J. *Prog. Brain Res.* **49**, 359–364 (1979).
11. Wigston, J. J. & Sanes, J. R. *Nature* **299**, 464–467 (1982).
12. Chevallier, A., Kiény, M. & Mauger, A. C. *J. Embryol. exp. Morph.* **41**, 245–258 (1977).
13. Christ, B., Jacob, H. J. & Jacob, M. *Anat. Embryol.* **150**, 171–186 (1977).
14. Duron, B., Marlot, D. & Macron, J. M. *Neurosci. Lett.* **15**, 93–96 (1979).

Enhancement of polyoma virus middle T antigen tyrosine phosphorylation by epidermal growth factor

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Polyoma virus codes for three proteins involved in host cell transformation: the large, middle and small T antigens. Middle T antigen is a major transforming protein which is responsible for the induction of the phenotype of transformed cells and, without it, transformation does not occur (reviewed in refs 1–4). Middle T antigen alone can transform established cell lines⁵, although large, and possibly small, T antigens are also required for the full expression of the phenotype of transformed cells in media with a low concentration of serum⁶. A subfraction of middle T antigen is associated with a protein kinase activity which phosphorylates middle T antigen *in vitro* on tyrosine^{7–11}. There is a strong correlation between the level of this kinase activity and the degree of expression of the phenotype of transformed cells^{7–9,12}. We report here that epidermal growth factor (EGF) stimulates tyrosine phosphorylation of middle T antigen, suggesting the possibility that mitogenic growth factor(s) regulates this phosphorylation activity.

Plasma membranes were purified from primary mouse embryo cells transformed by a mutant of polyoma virus, dl-8 (ref. 13), which is associated with a higher middle T antigen phosphorylating activity than the wild-type virus⁹. When this plasma membrane is incubated with [γ -³²P]ATP, a large number of cellular proteins become phosphorylated. If the plasma membrane is treated with EGF before the incubation with [γ -³²P]ATP, a phosphorylated protein with an apparent molecular weight of about 160,000 (160K) is easily detectable over the background of phosphorylated cellular proteins (Fig. 1A). Phosphorylation of the majority of cellular proteins are not affected by EGF treatment. Tyrosine is the main phosphate acceptor of the 160K phosphoprotein (Fig. 1B). These results are essentially the same as those described previously to indicate that the 160K protein is the EGF receptor^{14,15}. In Fig. 1, middle T antigen is buried in cellular proteins and, hence, undetectable. When middle T antigen is immunoprecipitated from the plasma membrane after incubation with [γ -³²P]ATP, approximately 2.7 times more phosphate was found incorporated into middle T antigen, mainly on tyrosine, in the EGF-treated plasma membrane than in untreated plasma membrane (Fig. 2). This increase of phosphorylation of middle T antigen is presumably due to the activation of tyrosine kinase associated with the EGF receptor¹⁴ by EGF. Weakly phosphorylated protein, which co-migrates with middle T antigen and precipitates with control serum, contains phosphoserine and a smaller amount of phosphothreonine (Fig. 2B, a). At least a part of the phosphoserine and phosphothreonine present in the middle T antigen preparation is therefore derived from this contaminating protein. It is uncertain whether the amounts of phosphoserine and phosphothreonine in middle T antigen are higher in the preparation from EGF-treated than from untreated plasma membrane.

It appears that the EGF stimulation enhances the level of tyrosine phosphorylation of middle T antigen (Fig. 2A, lane 3) but is not required for the basal level of middle T antigen phosphorylation seen in Fig. 2A, lane 1. This apparent EGF-independent phosphorylation of middle T antigen might be due to autophosphorylation or to the activity of other cellular tyrosine kinases. An obvious explanation for the increase of

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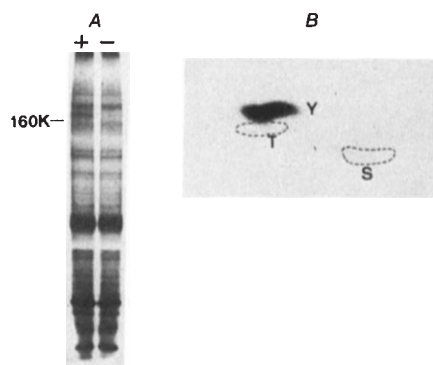


Fig. 1 Tyrosine phosphorylation of 160K protein after incubation, in the presence of EGF, of purified plasma membranes from mouse cells transformed by a polyoma virus mutant, dl-8 (dl-8/CBA cells). **A**, Purified plasma membranes from dl-8/CBA cells were incubated with (+) or without (-) EGF, and further incubated with [γ - 32 P]ATP. The samples were solubilized and analysed by 7.5% SDS-polyacrylamide gel electrophoresis. **B**, The 160K phosphorylated protein shown in **A** was eluted from the gel and phosphoamino acids were analysed. Y, phosphotyrosine; T, phosphothreonine; S, phosphoserine. Only very small amounts of phosphoserine and phosphothreonine were detected.

Methods: **A**, Isolation of plasma membranes. dl-8/CBA cells (obtained from G. Klein, Sweden)—primary mouse embryo fibroblasts from CBA strain transformed by a mutant of polyoma virus, dl-8¹³—were grown in plastic Petri dishes in Dulbecco's modified Eagles's minimum essential medium (DMEM) supplemented with 5% fetal bovine serum (FBS). The cells were washed once with phosphate-buffered saline (PBS) and twice more with PBS containing 1 mM EDTA. The cells were scraped off the dishes in cold PBS containing 5 mg ml⁻¹ bovine serum albumin (BSA) and pelleted by centrifugation at 1,500 r.p.m. for 5 min. The cells were suspended in 0.25 M sucrose, 10 mM Tris-HCl pH 7.4, 0.2 mM MgCl₂, 5 mg ml⁻¹ bovine serum albumin and disrupted by nitrogen cavitation (800 p.s.i.)^{17,20}. The disrupted cells were centrifuged at 31,000g for 20 min. The pellets were suspended in the sucrose buffer mentioned above, layered onto 25% Ficoll (w/v), and centrifuged at 100,000g for 9 h²¹. Materials at the interface were collected and suspended in 10 mM HEPES buffer pH 7.5 and used as a plasma membrane fraction. About 28 μ g of plasma membranes were incubated with or without 0.5 μ M EGF at 0°C for 15 min in 20 mM HEPES pH 7.4, 5 mM MgCl₂ (ref. 21) and were further incubated with 10 μ Ci of [γ - 32 P]ATP at a final concentration of 5 μ M for 10 min at 30°C. The samples were solubilized with SDS-containing electrophoresis sample buffer (10% glycerol, 5% β -mercaptoethanol, 2.3% SDS and 0.0625 M Tris-HCl pH 6.8) and analysed by 7.5% SDS-polyacrylamide gel electrophoresis as described previously²². **B**, Phosphoamino acid analysis. The 160K phosphorylated protein shown in **A** was eluted from the gel, hydrolysed in 6 M HCl for 2 h at 105°C. Phosphoamino acids were then analysed by two-dimensional electrophoresis—chromatography as described previously¹⁰.

phosphorylation of middle T antigen after EGF stimulation (Fig. 2A, lane 3) would be that middle T antigen is either directly phosphorylated by the EGF receptor kinase or indirectly via chain reactions involving one or more tyrosine kinases. If either were the case, middle T antigen would serve merely as a phosphate acceptor and at least a part of tyrosine phosphorylation of middle T antigen would be mediated by a cellular kinase. Alternatively, binding of EGF to its receptor might lead to activation of middle T antigen as a kinase which would autophosphorylate. Our earlier observation suggested that phosphorylation of unspecified protein is required for the middle T antigen phosphorylating activity¹⁰. The present results may suggest, therefore, that this phosphorylation required for the middle T antigen phosphorylating activity is provided by a cellular kinase(s) related to the EGF receptor kinase. It is not known, at present, whether this phosphorylation required for middle T antigen phosphorylating activity is tyrosine specific.

The increase of phosphorylation of the EGF receptor is much higher than that of middle T antigen after the EGF stimulation

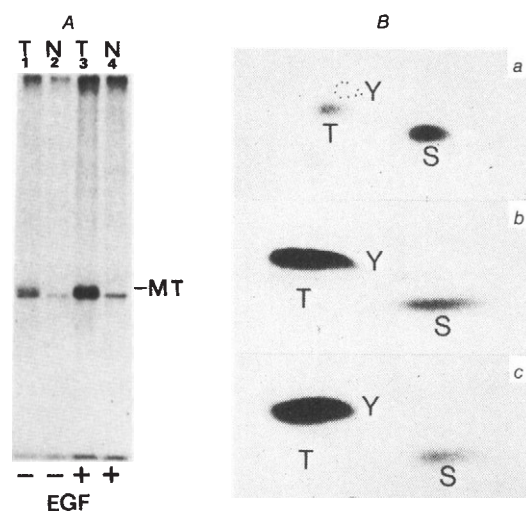


Fig. 2 Phosphorylation of middle T antigen in *in situ* phosphorylation reaction in the presence or the absence of EGF. **A**, *In situ* phosphorylated plasma membrane shown in Fig. 1 was solubilized with SDS. Middle T antigen was immunoprecipitated from this solubilized plasma membrane using rat anti-polyoma T antigen serum¹⁴ (T) or control normal rat serum (N). Immunoprecipitates were analysed by 10% SDS containing polyacrylamide gel electrophoresis. Lane 1, without EGF, control serum. Lane 2, without EGF, control serum. Lane 3, with EGF, anti-T serum. Lane 4, with EGF, control serum. MT, Middle T antigen. **B**, Phosphorylated proteins shown in **A** that migrate at or near the position marked MT were eluted from each of the four lanes. Since it was technically difficult, attempts were not made to take the region corresponding to middle T antigen separately from that of closely migrating background protein. Phosphoamino acids obtained from these proteins after acid hydrolysis were analysed as described in Fig. 1 legend. **a**, Material precipitated with normal rat serum, with EGF (lane 4 in **A**). **b**, Middle T antigen, without EGF (lane 1 in **A**). **c**, Middle T antigen, with EGF (lane 3 in **A**). The pattern of phosphoamino acids of the material precipitated with normal rat serum, without EGF (lane 2 in **A**), is similar to **a** (data not shown). Y, phosphotyrosine; T, phosphothreonine; S, phosphoserine. Phosphothreonine in **b** and **c** is detectable only on the original X-ray films.

Methods: Solubilization of membrane. *In situ* phosphorylated plasma membranes shown in Fig. 1 were solubilized with 3% SDS containing 100 mM EDTA at 90°C for 1 min and at room temperature for 30 min and diluted to 0.1% with respect to SDS with extraction buffer (100 mM NaCl, 100 mM Tris-HCl pH 8.0, 0.5% NP40, 100 kallikrein units ml⁻¹ of aprotinin (Sigma)) containing 100 mM EDTA. Middle T antigen was immunoprecipitated from this solubilized plasma membrane.

(Figs 1, 2). This tends to suggest that middle T antigen is unlikely to be a direct substrate of the EGF receptor kinase.

This increase of tyrosine phosphorylation of middle T antigen after EGF stimulation could also occur if EGF stimulation were to lead to a decrease in activity of a protein phosphatase specific for a small number of phosphoproteins, including middle T antigen. It has been shown that calf intestine alkaline phosphatase removes phosphate groups from *in vitro* phosphorylated middle T antigen¹⁰. We have confirmed that the EGF used in the present studies does not have an inhibitory or stimulatory effect on calf intestine alkaline phosphatase in the conditions used in the experiment shown in Fig. 1 (data not shown).

In any event, it is important to note that middle T antigen appears to be in close association not necessarily physically but functionally with the EGF receptor which is located in the plasma membrane¹⁶. The present results provide an independent support, although indirectly, for the notion that a subpopulation of middle T antigen is in the plasma membrane^{9,10,17}.

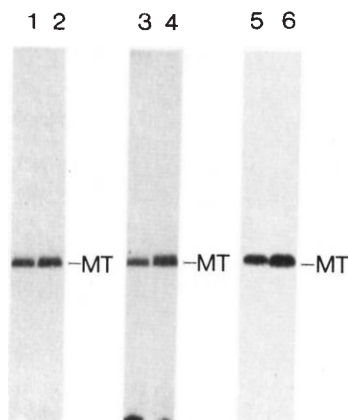


Fig. 3 Effect of EGF on the middle T antigen phosphorylating activity in cells expressing only middle T antigen. The clone 2-4 of Rat-1 cells transformed by an altered polyoma virus DNA capable of inducing only middle T antigen⁵ (obtained from R. Kamen) was grown to confluency in DMEM supplemented with 10% fetal bovine serum (FBS). The cells were then incubated for 15 h in DMEM containing 0.5% FBS. At the end of this period the cells were incubated for 1 h, before cell extracts were made, in the following media: DMEM with (lane 2) or without (lanes 1, 3, 5) 100 ng ml⁻¹ EGF or DMEM containing 20% FBS (lane 4) or conditioned medium (lane 6). Middle T antigen was immunoprecipitated from these cell extracts, subjected to *in vitro* phosphorylation reaction and analysed by 10% polyacrylamide gel electrophoresis. MT: middle T antigen. The experiments shown in the three panels were not performed simultaneously. The conditioned medium used in these experiments was DMEM without serum incubated for 2 days with T1A1 cells (primary rat embryo cells transformed by wild-type polyoma virus¹⁷).

When EGF was added to the culture of intact dl-8/CBA cells, no clearcut enhancement of either *in vivo* phosphorylation of middle T antigen or *in vitro* middle T antigen phosphorylating activity was seen (data not shown). It is conceivable that dl-8/CBA cells secrete some mitogenic growth factor(s) that obscures the effect of added EGF (see below). It has been reported that, unlike ordinary transformed cells, the cells expressing only middle T antigen have the high saturation density characteristic of transformed cells only when they are maintained in medium containing a high concentration of serum, suggesting that a function of middle T antigen might be enhanced by externally added growth factors⁶. When such cells that express only middle T antigen are starved for serum for 15 h and are then treated with EGF, fresh serum or conditioned medium, middle T antigen phosphorylating activity is enhanced (Fig. 3, Table 1). Although small, this increase of middle T antigen phosphorylating activity was also observed with two other clones of rat cells expressing only middle T antigen (data not shown). It is not known whether the number of EGF receptors on the cells increases after these treatments. The conditioned medium used here was a serum-free culture supernatant of polyoma virus-transformed rat cells. Similarly obtained conditioned medium was shown to contain transforming growth factor¹⁸. The fact that the conditioned medium also has stimulatory effect on middle T antigen phosphorylating activity suggests that an active principle in the medium may be a transforming growth factor(s).

It has recently been reported that pp60^{c-src}, the cellular homologue of the transforming protein of avian sarcoma virus, is in tight association with a subfraction of middle T antigen, suggesting that tyrosine phosphorylation of middle T antigen observed *in vitro* is mediated by pp60^{c-src}, a tyrosine kinase¹⁹. Our present results are consistent with this report in the sense that a known cellular tyrosine kinase appears to be responsible for at least a part of middle T antigen phosphorylating activity. If such complexes are also formed *in vivo*, a basal level of tyrosine phosphorylation of middle T antigen detectable before

Table 1 Effects of EGF, FBS or conditioned medium on *in vitro* middle T antigen phosphorylating activity

	Mitogen	Treated (c.p.m.)	Untreated (c.p.m.)	Ratio treated/untreated
<i>In vitro</i> middle T antigen phosphorylating activity	EGF (100 ng ml ⁻¹)	3,563	2,070	1.72
	FBS (20%)	3,379	1,736	1.94
	Conditioned medium	6,484	4,418	1.46

The areas of phosphorylated middle T antigen in the gels shown in Fig. 3 were cut, dissolved in H₂O₂ and the radioactivities were counted in Aquasol (NEN).

the EGF treatment (Fig. 2A, lane 1) might be due to a pp60^{c-src} kinase. It would then be interesting to explore the possibility that EGF would stimulate pp60^{c-src} kinase activity.

The level of middle T antigen phosphorylating activity, as measured by the method shown in the experiment in Fig. 3, correlates well with the ability of the virus to induce the phenotype of transformed cells. In this regard, it will be of interest to study the effects of various externally added mitogenic growth factors on middle T antigen phosphorylating activity.

Received 3 May; accepted 5 July 1983.

1. Ito, Y. in *Viral Oncology* (ed. Klein, G.) 447-480 (Raven, New York, 1980).
2. Eckhart, W. *Adv. Cancer Res.* **35**, 1-25 (1981).
3. Griffin, B. E. & Dilworth, S. M. *Adv. Cancer Res.* **39**, (in the press).
4. Benjamin, T. L. *Biochim. biophys. Acta* **695**, 69-95 (1982).
5. Treisman, R., Novak, U., Favaloro, J. & Kamen, R. *Nature* **292**, 595-600 (1981).
6. Rassoulzadegan, M. *et al. Nature* **300**, 713-718 (1982).
7. Eckhart, W., Hutchinson, M.-A. & Hunter, T. *Cell* **18**, 925-933 (1979).
8. Schaffhausen, B. S. & Benjamin, T. L. *Cell* **18**, 935-946 (1979).
9. Smith, A. E., Smith, R., Griffin, B. E. & Fried, M. *Cell* **18**, 915-924 (1979).
10. Segawa, K. & Ito, Y. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6812-6816 (1982).
11. Walter, G., Hutchinson, M.-A., Hunter, T. & Eckhart, W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4025-4029 (1982).
12. Ito, Y., Spurr, N. & Griffin, B. E. *J. Virol.* **35**, 219-232 (1980).
13. Griffin, B. E. & Maddock, C. J. *J. Virol.* **31**, 645-656 (1979).
14. Cohen, S., Carpenter, G. & King, L. Jr. *J. biol. Chem.* **255**, 4834-4842 (1980).
15. Ushiro, H. & Cohen, S. *J. biol. Chem.* **255**, 8363-8365 (1980).
16. Carpenter, G., King, L. Jr. & Cohen, S. *Nature* **276**, 409-410 (1978).
17. Ito, Y., Brocklehurst, J. R. & Dulbecco, R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4666-4670 (1977).
18. Kaplan, P. & Ozanne, B. *Virology* **123**, 372-380 (1982).
19. Courtneidge, S. A. & Smith, A. E. *Nature* **303**, 435-439, (1983).
20. Nishimura, J., Huang, J. S. & Deuel, T. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4303-4307 (1982).
21. Whittenberger, G. & Glaser, L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2251-2255 (1977).
22. Ito, Y. *Virology* **98**, 261-266 (1979).

Sequences homologous to episomal mitochondrial DNAs in the maize nuclear genome

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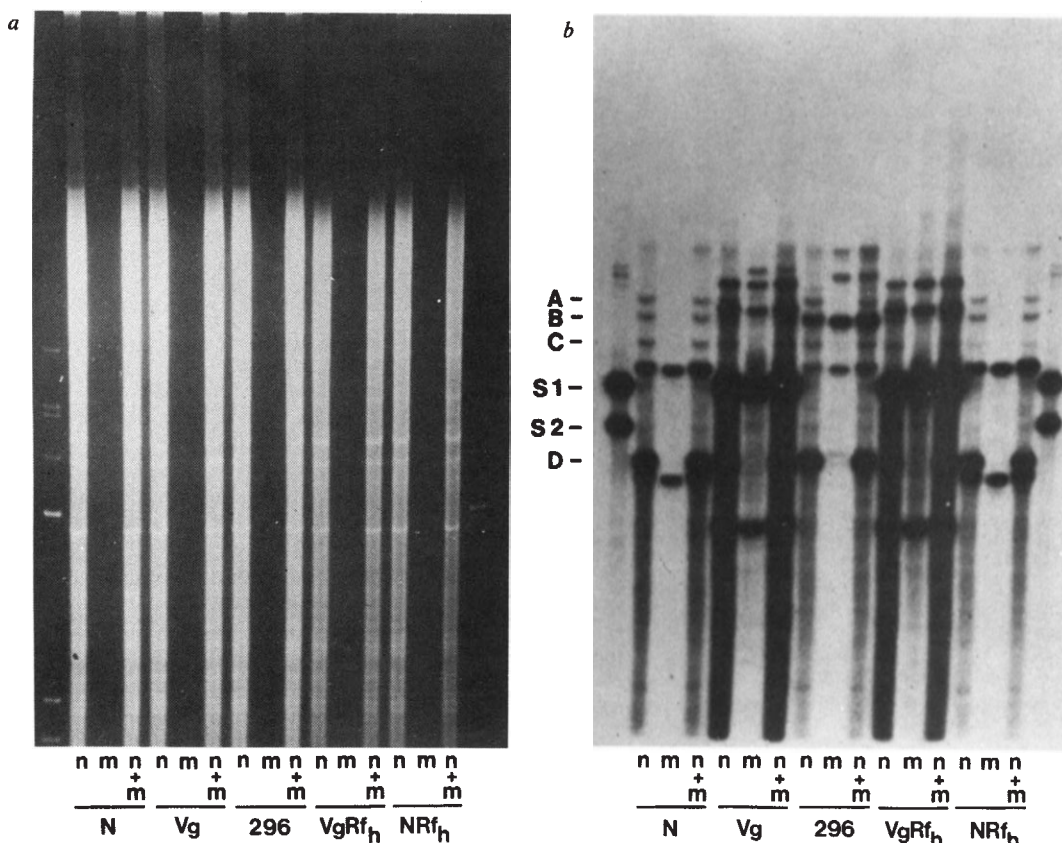
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The endosymbiont hypothesis for the origin of eukaryotic cells implies that an exchange of genetic information between nuclei, mitochondria and, in the case of plants, chloroplasts is plausible. Indeed, a 12-kilobase (kb) DNA sequence common to chloroplasts and mitochondria of maize has been identified¹ and we now report the presence of common DNA sequences in the maize nuclear and mitochondrial genomes. Homologous nuclear and mitochondrial DNA (mtDNA) sequences have also

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Fig. 1 Localization of S-1 and S-2 sequences among electrophoretically separated *Bam*HI restriction fragments of nuclear and mtDNAs from male sterile and fertile maize. **a** Shows an ethidium bromide stained 1% agarose gel of nuclear DNA (n), mtDNA (m) and a mixture of both (n+m) from: N, M825/Oh07(N); Vg, parental M825/Oh07(Vg); 296, cytoplasmic revertant to fertility 296; VgRf_h, nuclear revertant M825/Oh07Rf_h(Vg); NRf_h, nuclear revertant M825/Oh07Rf_h(N). The two outermost lanes contain a low concentration of *cms*-S mtDNA and DNA size markers generated by independent digestions of λ DNA with *Eco*RI and *Hae*III. **b** Is an autoradiograph of a Southern blot of the gel after hybridization with a mixed probe of pZmS21 and pZmS4. The positions of S-1, S-2 and nucleus-specific DNA fragments A-D are indicated.



Methods: mtDNA was isolated from dark-grown coleoptiles as described by Kemble *et al.*²⁵. Nuclear DNA was isolated from similar tissue by grinding for 30 s at 4 °C in a mortar and pestle containing homogenization buffer (0.3 M sucrose, 50 mM Tris-HCl pH 7.4, 5 mM magnesium chloride). The homogenate was filtered through four layers of cheesecloth before centrifugation at 700g for 10 min. The pellet was gently resuspended in homogenization buffer and 20% Triton X-100 was added to a final concentration of 1%, before recentrifuging at 700g for 10 min. This resuspension and recentrifugation step was repeated once more. The pellet was gently resuspended in homogenization buffer containing 40% metrizamide and layered onto a discontinuous step gradient of 40% and 50% metrizamide in homogenization buffer. After centrifugation at 36,000g for 20 min the band appearing at the 40%:50% metrizamide interface was recovered and slowly diluted with homogenization buffer. The nuclei in the preparation were sedimented at 3,000g for 10 min and lysed in 50 mM Tris-HCl pH 8.0, 10 mM EDTA, 2% sarkosyl and 0.012% proteinase K at 65 °C for 5 min followed by 37 °C for 1 h. Nuclear and mtDNA was further extracted in the same manner and either subjected to electrophoresis without additional treatment or initially fragmented with restriction endonucleases²⁵. DNA in gels was transferred to nitrocellulose²⁶ and prehybridized for 14–24 h at 42 °C according to Wahl *et al.*²⁷. Hybridizations, at 42 °C, contained dextran sulphate²⁷ and 1–2 × 10⁸ c.p.m. freshly boiled nick-translated²⁸ cloned DNA. The nitrocellulose was washed three times in 2 × SSC, 0.1% SDS at room temperature for 5 min each, followed by four washes of 0.1 × SSC, 0.1% SDS at 50 °C for 15 min each. Autoradiography was performed at –70 °C using intensifying screens. Reconstitution experiments indicated that our hybridization conditions were capable of detecting between one and two copies of S-1 and S-2 sequences.

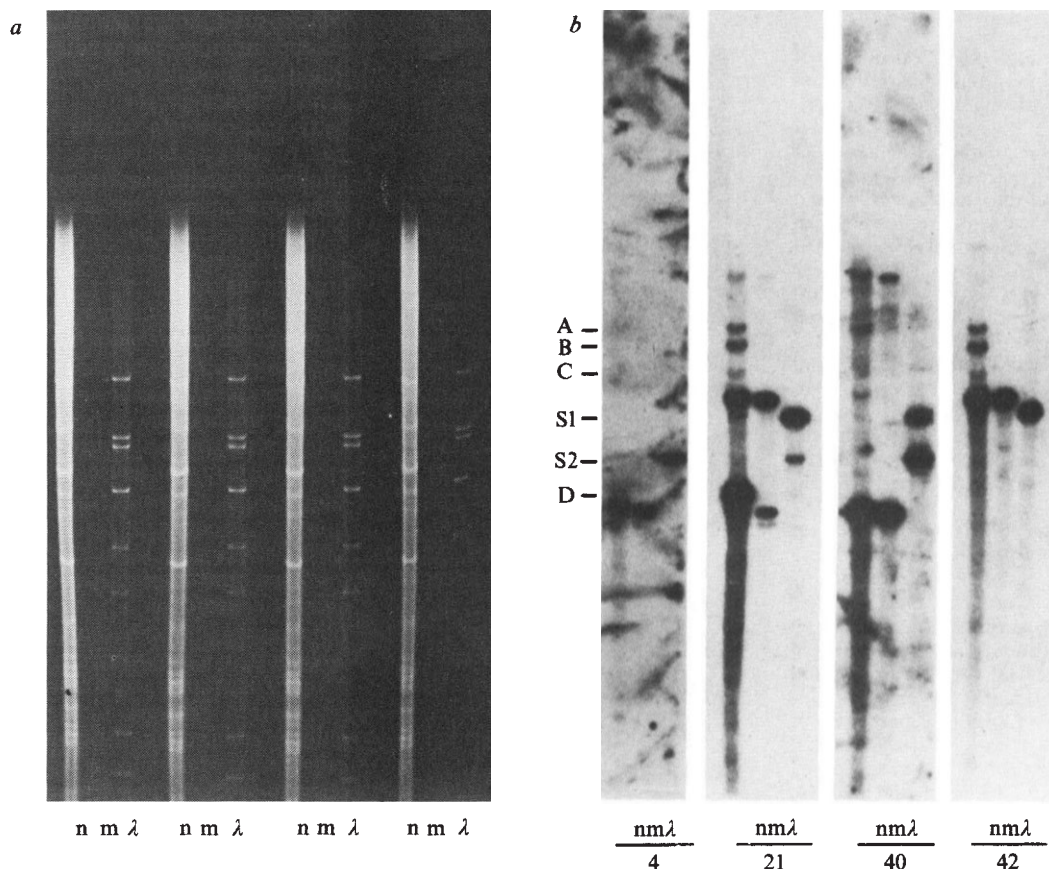
recently been found in other organisms^{2–6}, but this report extends the phenomenon to a higher eukaryote. Moreover, it encompasses sequences common to the mitochondrial genome, the nuclear genome and episomal, virus-like DNAs that have the physical characteristics of transposable elements. These episomal DNAs may provide the mechanism whereby sequences are transported from the mitochondrion to the nucleus.

In maize there are three groups of cytoplasm capable of causing cytoplasmic male sterility (*cms*), providing that the nucleus does not possess group-specific male fertility restorer (*Rf*) genes which override the *cms* effect. These three groups are designated S, C and T, whereas a male fertile cytoplasm is designated N. Some *cms*-S maize lines spontaneously revert to male fertility; for example, M825/Oh07 lines containing Vg cytoplasm (a member of the *cms*-S group) revert at a frequency of about 11%. Using these revertants to male fertility in subsequent crossing programmes, it was determined that some plants reverted due to nuclear mutations (termed nuclear revertants), whereas in others the mutations were cytoplasmic (termed cytoplasmic revertants)^{7–9}. On the basis of such genetic data, an episomal 'fertility element' which could be 'fixed' in either the cytoplasm or the nucleus was postulated as the molecular component responsible for these reversions¹⁰.

Among the extrachromosomal DNAs present in maize mitochondria¹¹, the best candidates for the role of fertility element were S-1 and S-2, linear episomes of 6.2 and 5.2 kb, respectively. They are present only in the mitochondria of *cms*-S group lines¹² and resemble transposable elements by possessing terminal inverted repeats and additional regions of sequence homology¹³. They possess a protein covalently attached to their 5' termini¹⁴, and therefore resemble adenovirus, the terminal protein of which may prime replication of the molecules^{15–17}. Potential replicative forms of S-1 and S-2 have been identified¹⁸. It has been suggested that S-1 and S-2 evolved by excision from the mitochondrial genome of a N, male fertile, cytoplasm line^{19–21} to give rise to *cms*-S lines in which they are amplified as virus-like DNAs. The first direct evidence that S-1 and S-2 could be the postulated fertility elements was found in M825/Oh07(Vg) cytoplasmic revertants to fertility. In these strains, S-1 and S-2 are not present as virus-like DNAs but have either transposed into the mitochondrial chromosome²² or, alternatively, homologous sequences in the mitochondrial chromosome have preferentially become amplified and rearranged¹⁸.

As the fertility element hypothesis predicts that S-1 and S-2 sequences would be transposed into the nuclear DNA of nuclear revertants, we analysed this by hybridizing restriction fragments

Fig. 2 Extent of S-1 represented among *Bam*HI fragments of nuclear and mtDNAs of a nuclear revertant to fertility. *a* Shows quadruplicate ethidium bromide stained gels of *Bam*HI digests of nuclear (n) and mitochondrial (m) DNA from the nuclear revertant to fertility M825/Oh07Rf/h(N). Lanes λ contain DNA markers as in Fig. 1. *b* Is an autoradiograph of a Southern blot of the gel after separating the four sets of lanes and hybridizing each to a different cloned probe. Sets of lanes labelled 4, 21, 40 and 42 were hybridized to pZmS4 (contains sequences unique to S-2), pZmS21 (contains unique S-1 sequences and sequences homologous to S-2), pZmS40 (contains unique S-2 sequences and sequences homologous to S-1) and pZmS42 (contains unique S-1 sequences), respectively. Other labelling is as in Fig. 1.



of nuclear DNA, isolated from the *cms* parental lines and the nuclear revertants, with cloned probes of S-1 and S-2 described previously^{18,19} (see Fig. 2 legend). For each maize line, we analysed nuclear DNA, mtDNA in a concentration similar to the amount present as a contaminant in the nuclear sample, and a combination of these two samples (Fig. 1). Because S-1 and S-2 remain as extrachromosomal DNAs in the mitochondria of nuclear revertants, it is difficult to detect low copy number, nucleus-specific bands on the autoradiograph (Fig. 1b). Therefore, to facilitate the analysis, these plants were crossed as pollen donors with their N cytoplasm maintainer line as the female parent, to produce isogenic lines carrying N cytoplasm. There are four nuclear DNA-specific fragments homologous to the S-1 and S-2 probes in the nuclear revertants analysed (Fig. 1b). These fragments, labelled A-D, are 10.2, 8.9, 7.6 and 4.7 kb, respectively. However, they are also present in the parental lines and in the cytoplasmic revertant analysed, indicating that their presence is not due to transposition of S-1 and S-2 associated with the observed reversion. The fragments cannot be due to mtDNA contamination of the nuclear DNA because they are not detected in mtDNA preparations (Fig. 1b). Also, if they were due to contamination, their mobility would be different in the N, Vg and cytoplasmic revertant lanes because the mtDNA organization of S-1 and S-2 sequences differs in each¹⁸.

Hybridization experiments using individual pZmS clones (Fig. 2) indicated that fragment A possibly represents a full-length copy of S-1. A large region of S-1, but lacking the region of homology, may be represented in fragments B and C. Only the central region of S-1 is present in fragment D. If the initial molecular event was insertion of a complete copy of S-1 into the nuclear genome (which fragment A could represent), genomic mutations and rearrangements must have occurred to give rise to the other fragments. In this and other experiments, using different restriction enzymes, no fragments hybridized with pZmS4, suggesting that sequences unique to S-2 are not found in nuclear DNA.

The three nuclear revertants analysed all produced the same hybridization pattern (Fig. 3, lanes 1-3). Small nucleus-specific

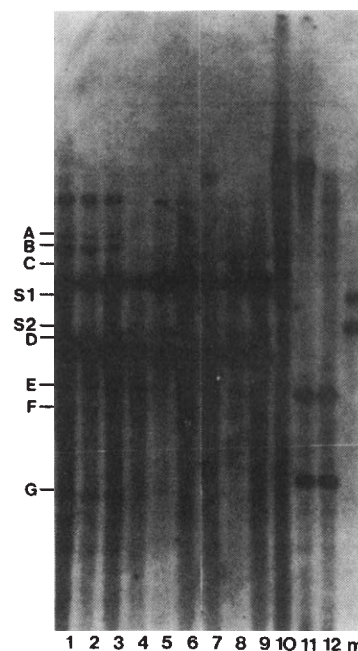


Fig. 3 Varying degrees of S-1 integration into nuclear DNA in different maize lines. Autoradiograph of a 1% agarose gel after transfer to nitrocellulose and hybridization to a mixed probe of pZmS21 and pZmS4. The gel contained *Bam*HI fragmented nuclear DNA from the nuclear revertants M825/Oh07Rf/h(N), M825/Oh07Rf/i(N) and M825/Oh07Rf/m(N) in lanes 1, 2 and 3, respectively; *Bam*HI fragmented nuclear DNA, mtDNA and a mixture of both from *TrRf3Rf3*(N) line in lanes 4, 5 and 6, respectively; *Bam*HI fragmented nuclear DNA, mtDNA and a mixture of both from W64A(N) inbred line in lanes 7, 8 and 9, respectively; nuclear DNA from the nuclear revertant M825/Oh07Rf/m(N) not subjected to restriction enzymes in lane 10, fragmented with *Hpa*II in lane 11 and with *Msp*I in lane 12. Lane m contained DNA size markers as in Fig. 1.

fragments, E (3.65 kb), F (3.3 kb) and G (2.4 kb), were visible as distinct bands (visible as diffuse bands in lines analysed in Fig. 1). They do not hybridize with pZmS4 alone, again suggesting no involvement of sequences unique to S-2. Because nuclear revertants have apparently acquired new male fertility restorer genes, it was suggested that the fertility element may be analogous in structure to *Rf3*, the standard *cms-S* nuclear restorer gene⁷. If this is correct and S-1 and S-2 are the postulated fertility elements, fragments hybridizing to S-1 and S-2 would be present in nuclear revertants and *Rf3* lines but absent in lines lacking restorer genes. However, this is not observed. The only nucleus-specific hybridizing fragment present in both the *TrRf3Rf3* line and the inbred W64A, which does not possess *Rf3*, is fragment D (Fig. 3, lanes 4–9). B37, another inbred line lacking *Rf3*, exhibits the same hybridization pattern (data not shown). Figure 3, lane 10, indicates that the S-1 sequences observed are integrated into the nuclear DNA because no bands corresponding to the mobility of virus-like S-1 and S-2 DNAs are visible. Lanes 11 and 12 confirm many earlier reports that plant nuclear DNA is highly methylated.

Although the data suggest that S-1 and S-2 are not themselves the postulated fertility elements, they demonstrate homologous sequences between the nuclear genome, the mitochondrial chromosome and virus-like extrachromosomal mtDNAs. Such a DNA transfer could have occurred by S-1 and S-2 becoming excised from a N cytoplasm mitochondrial genome¹⁹ to give rise to the virus-like conformations which are maintained and amplified. S-1 may have left the mitochondrion and entered the nucleus where it was transposed into the genome. This event may have occurred only once in the evolution of maize. Since that time, preferential genomic amplifications, excisions and rearrangements may have altered the copy number of the S-1 homologous sequences to account for the differences in strains such as M825/Oh07, W64A and *Tr*. Sequence promiscuity²³ may account for the presence of S-1 sequences in the nucleus and their reorganizational activities in the mitochondrion of *cms* lines¹⁸.

This possible movement of genetic information from the mitochondrion to the nucleus via these virus-like DNAs may not be entirely unique, as we have also detected nuclear DNA sequences homologous to another maize mitochondrial extrachromosomal DNA, the 1.94-kb plasmid¹¹ (data not shown). Again, the nuclear DNA fragments exhibiting hybridization to this plasmid differ between lines.

S-1 and S-2 have been proposed as potential vehicles for plant transformation studies²⁴. The finding of S-1 sequences in nuclear DNA increases this potential since it may be possible to integrate foreign DNA into the genome by both transposition and recombination of the homologous sequences. It may be possible to perform similar experiments with the 1.94-kb mitochondrial plasmid.

We thank Dr J. E. Carlson for helpful discussions. This work was supported in part by the United States Department of Energy, Division of Biological Energy Research, report number DOE/ET/010904-2 (to R.J.M.) and DE-AC02-81ER10873 (to J.R.L.). Kansas Agricultural Experiment Station Contribution no. 83-107-J.

Received 29 March; accepted 15 June 1983.

1. Stern, D. B. & Lonsdale, D. M. *Nature* **299**, 698–702 (1982).
2. Farrelly, F. & Butow, R. A. *Nature* **301**, 296–301 (1983).
3. Wright, R. M. & Cummings, D. J. *Nature* **302**, 86–88 (1983).
4. Jacobs, H. T. *et al. J. molec. Biol.* **165**, 609–632 (1983).
5. Gellissen, G., Bradfield, J. Y., White, B. N. & Wyatt, G. R. *Nature* **301**, 631–634 (1983).
6. van den Boogaart, P., Samallo, J. & Agsteribbe, E. *Nature* **298**, 187–189 (1982).
7. Laughnan, J. R. & Gabay, S. J. *Theor. appl. Genet.* **43**, 109–116 (1973).
8. Singh, A. & Laughnan, J. R. *Genetics* **71**, 607–620 (1972).
9. Laughnan, J. R., Gabay-Laughnan, S. & Carlson, J. E. *Stadler Symp.* **13**, 93–114 (1981).
10. Laughnan, J. R. & Gabay, S. J. in *Maize Breeding and Genetics* (ed. Walden, D. B.) 427–446 (Wiley, New York, 1978).
11. Kemble, R. J. & Bedbrook, J. R. *Nature* **284**, 565–566 (1980).
12. Pring, D. R., Levings, C. S. III, Hu, W. W. L. & Timothy, D. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2904–2908 (1977).
13. Kim, B. D., Mans, R. J., Conde, M. F., Pring, D. R. & Levings, C. S. III *Plasmid* **7**, 1–14 (1982).
14. Kemble, R. J. & Thompson, R. D. *Nucleic Acids Res.* **10**, 8181–8190 (1982).
15. Rekosh, D. M. K., Russell, W. C., Bellett, A. J. D. & Robinson, A. J. *Cell* **11**, 283–295 (1977).

16. Challberg, M. D., Desiderio, S. V. & Kelly, T. J. Jr. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5105–5109 (1980).
17. Tamanoi, F. & Stillman, B. W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2221–2225 (1982).
18. Kemble, R. J. & Mans, R. J. *J. molec. appl. Genet.* **2**, 161–171 (1983).
19. Thompson, R. D., Kemble, R. J. & Flavell, R. B. *Nucleic Acids Res.* **8**, 1999–2008 (1980).
20. Lonsdale, D. M., Thompson, R. D. & Hodge, T. P. *Nucleic Acids Res.* **9**, 3657–3669 (1981).
21. Koncz, C., Simegi, J., Udvardy, A., Racsmany, M. & Dudits, D. *Molec. gen. Genet.* **183**, 449–458 (1981).
22. Levings, C. S. III *et al. Science* **209**, 1021–1023 (1980).
23. Ellis, S. *Nature* **299**, 678–679 (1982).
24. How, S. H. A. *Rev. Pl. Physiol.* **33**, 609–650 (1982).
25. Kerle, R. J., Gunn, R. E. & Flavell, R. B. *Genetics* **95**, 451–458 (1980).
26. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
27. Wahl, G. M., Stern, M. & Stark, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
28. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184–1188 (1975).

Stimulation of transcription of the yeast tRNA^{Tyr} gene in cell-free extracts by tyrosyl-tRNA synthetase

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Eukaryotic transfer RNA genes have two internal discontinuous control regions, the A and B blocks, which correspond approximately to the D-stem and the pseudo-U arm of tRNA^{Lys}. In reconstituted transcription systems at least two components are required to direct accurate initiation by RNA polymerase (refs 4, 5). However, little is known about the mechanism of interaction of the internal promoter sequences with factors and RNA polymerase C within the transcription complex, although tRNA-like conformation of the B block sequence was surmised to be critical for DNA recognition⁶. By analogy with the 5S RNA system, where a transcription factor required for 5S DNA expression was shown to interact both with 5S RNA^{7,8} and with the noncoding strand of the 5S gene⁹, we explored the possibility that a protein which normally binds to tRNA could also interact with the tRNA gene and regulate its transcription. Here we show that *in vitro* transcription of the yeast *SUP4* tRNA^{Tyr} gene in crude yeast extracts is strongly stimulated by tyrosyl-tRNA synthetase (TyrRS) but not by two other non-cognate synthetases. Substrates of the synthetase, tRNA^{Tyr} and tyrosine, interfere with stimulation of tRNA synthesis.

Recently, crude yeast extracts were developed for the DNA-dependent specific transcription of 5S RNA and several yeast tRNA genes^{10,11}. Transcription of the yeast *SUP4* tRNA^{Tyr} gene in such crude extracts yields a 110-nucleotide pre-tRNA^{Tyr} with small amounts of maturation products of 98 and 78 nucleotides¹¹. Figure 1 shows that the addition of purified TyrRS to transcription mixtures containing a low extract concentration resulted in a sevenfold increase in the amount of pre-tRNA^{Tyr} transcript (Fig. 1, lanes 1–4). Neither yeast phenylalanyl-tRNA synthetase (lane 6) nor seryl-tRNA synthetase from sheep liver (lane 7) or from yeast (not shown) significantly influenced the transcription of the tRNA^{Tyr} gene. Tyrosyl-tRNA synthetase only slightly increased transcription of a yeast tRNA^{Ser} gene (lanes 8, 9) and did not affect yeast 5S RNA gene transcription (result not shown). On the other hand, there was no specific transcription of the tRNA^{Tyr} gene by purified yeast RNA polymerase C in various ratios with TyrRS (result not shown).

The increased accumulation of pre-tRNA^{Tyr} in the presence of exogenous tRNA synthetase could be due to a specific protection of the synthesized tRNA against nucleolytic activities

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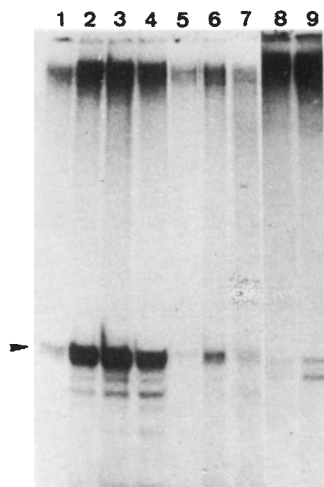


Fig. 1 The effect of aminoacyl-tRNA synthetases on transcription of yeast tRNA^{Tyr} and tRNA^{Ser} genes *in vitro*. The cell-free extract was prepared from *Saccharomyces cerevisiae* RNase 3⁻ strain as described by Koski *et al.*¹¹, including the gel filtration step on Sephadex G-25. The recombinant plasmids used as template were pPM16 produced by insertion in pBR322 of a 1.1-kilobase (kb) fragment of yeast DNA containing the suppressor *SUQ5* tRNA^{UAA} gene¹⁶ and pPM25, containing a 4.1-kb insert of yeast DNA with the suppressor *SUP4-O* tRNA^{Tyr} gene¹⁷ cloned in pBR322¹¹. Transcription reactions were carried out in 40-μl mixtures containing 20 mM HEPES-HCl pH 7.5, 5 mM MgCl₂, 70 mM KCl, 0.1 mM dithiothreitol 10% glycerol, 0.6 mM ATP, GTP, CTP and 0.03 mM [α -³²P]UTP (10 Ci mmol⁻¹), 5 μg ml⁻¹ plasmid DNA and the Sephadex G-25 fraction (75 μg ml⁻¹ protein). After 60 min incubation at 30 °C, transcription was stopped by adding 100 μl of solution containing 5 μg of carrier yeast tRNA, 50 mM sodium acetate pH 5, and 0.5% SDS. RNA was extracted with a phenol-chloroform mixture, ethanol precipitated and subjected to electrophoresis on polyacrylamide gel containing 7 M urea¹¹. Labelled RNA bands were revealed by autoradiography with Kodak X-OMAT S film without intensifying screen. *SUP4* tRNA^{Tyr} gene was present in expts 1–7, *SUQ5* tRNA^{Ser} gene in lanes 8 and 9. The experiment in lane 1 was the control transcription mixture. Other transcription mixtures were supplemented as follows with yeast tyrosyl-tRNA synthetase¹² in lanes 2 (0.1 μg), 3 (0.2 μg), 4, 5 and 9 (0.5 μg in each); sheep liver seryl-tRNA synthetase (0.5 μg) in lane 6; yeast phenylalanyl-tRNA synthetase (0.5 μg) in lane 7; yeast tRNA^{Tyr} (ref. 12) (0.8 μg) in lane 5.

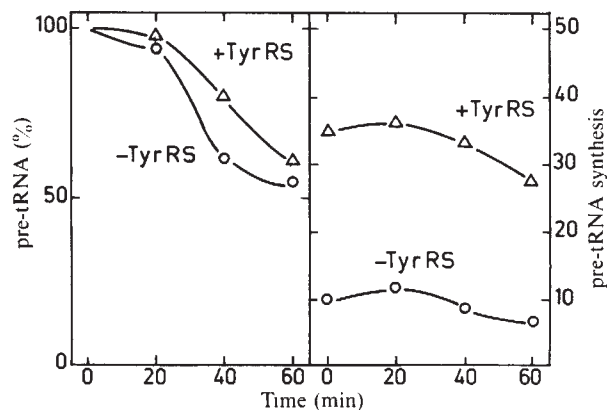


Fig. 2 Effect of tyrosyl-tRNA synthetase on the stability of synthesized pre-tRNA^{Tyr}. Left: *in vitro* transcription of the tRNA^{Tyr} gene was carried out as described in Fig. 1 legend in a 400 μl mixture. ³²P-labelled RNA was phenol extracted, ethanol precipitated and incubated in 160 μl with the same transcription buffer containing the unlabelled nucleoside triphosphates, 40 μg of G-25 protein fraction, with (Δ) or without (○) 2 μg of tyrosyl-tRNA synthetase. Aliquots (40 μl) were drawn at different times to analyse the RNA by electrophoresis, as in Fig. 1. The pre-tRNA band was quantified by scanning the autoradiogram with a Vernon densitometer and the peak area is given in arbitrary units. Right: Two transcription mixtures (200 μl), prepared as above, with (Δ) or without (○) 2.5 μg tyrosyl-tRNA synthetase were incubated for 1 h at 30 °C, then supplemented with 2 mM unlabelled UTP and further incubated. At different times, the RNA was analysed by electrophoresis and the pre-tRNA band estimated by densitometry as indicated above. It was checked that 2 mM unlabelled UTP, added at zero time, suppressed the incorporation of ³²P-labelled UMP into RNA.

of the extract. To investigate the effect of TyrRS on the stability of the transcript, the ³²P-labelled pre-tRNA was phenol extracted from the transcription mixture and incubated with low or high extract concentrations, in the presence or absence of TyrRS. In both cases, the half life of the labelled RNA was about 1 h (Fig. 2, left). Pulse-chase experiments also indicated that TyrRS, at the concentration used for stimulation, did not significantly protect the RNA (Fig. 2, right).

Figure 3 shows the dose-response curve of specific tRNA synthesis as a function of TyrRS concentration, at two extract concentrations. At low extract concentration, an eightfold stimulation was obtained with 0.3 μM TyrRS (molecular weight 80,000)¹² which was the optimal concentration. Stimulation decreased when more synthetase was added (Fig. 3), and this trend could not be reversed by doubling the DNA concentration (data not shown). At higher extract concentration, the extent of stimulation was more significant—16-fold with 0.5 μM TyrRS—and there was no decrease in specific transcription with an excess of synthetase. In fact, the optimal concentration of TyrRS seemed to shift with the extract concentration.

We investigated the effect of ligands of tyrosyl-tRNA synthetase on the transcription system. Specific transcription of tRNA^{Tyr} gene by the crude extract without synthetase was only slightly affected by tRNA^{Tyr} (3 μM) or tyrosine (60 μM) (Fig. 4). In contrast, when transcription was carried out in the pres-

ence of TyrRS, the addition of tRNA^{Tyr} (0.8 μM) produced a decrease of the pre-tRNA signal to the basal level of the non-stimulated control signal (Fig. 1, lane 5, and Fig. 4). tRNA^{Tyr} inhibited transcription by 50% when added at a 0.5:1 M ratio with tyrosyl-tRNA synthetase. Tyrosine (60 μM) prevented the stimulation by TyrRS while having little influence on tRNA synthesis by the extract alone (Fig. 4). The effect of tyrosine was specific, as several other amino acids—phenylalanine, methionine, threonine, serine and alanine—did not affect stimulation. The unusual nucleotide Ap₄A, a back-reaction product of the amino acid activation step¹³, which is produced with varying efficiency by most prokaryotic and eukaryotic synthetases¹⁴, did not influence the transcription of tRNA gene in the crude extract, with or without synthetase, at a concentration range of 1 nM–10 μM Ap₄A (results not shown).

Several mechanisms could be envisaged to account for the observed *in vitro* stimulation of tRNA synthesis by the cognate aminoacyl-tRNA synthetase. The fact that tRNA interferes with the stimulation suggests that the tRNA binding site of the enzyme interacts with a component of the transcription complex, either the nascent pre-tRNA or the noncoding strand of DNA. Binding of TyrRS to the nascent tRNA could favour its dissociation and increase the turnover rate of RNA polymerase. Alternatively, if the noncoding strand of the tRNA gene folds into a tRNA-like structure during transcription, it may be recognized by the synthetase which could thereby help to maintain the template in the active configuration. Binding of the enzyme to the double-stranded template is another possibility, as alanyl-tRNA synthetase was found to bind to a specific DNA sequence and repress its own gene transcription¹⁵. However, in preliminary experiments we found no specific binding of TyrRS to the restriction fragment bearing the *SUP4* gene. Experiments now in progress are aimed at determining whether TyrRS interacts with pre-tRNA^{Tyr} and/or with the coding or noncoding strand of the tRNA gene which contains a 14-base pair intron¹⁶. The mode of action of TyrRS will be best investigated in a purified transcription system. Recently, using a reconstituted system, with purified RNA polymerase C and two

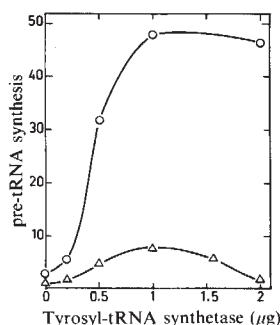


Fig. 3 Synthesis of tRNA^{Tyr} at different tyrosyl-tRNA synthetase concentrations. Transcription mixtures (40 μ l) were as described in Fig. 1 legend, with 5 μ g (Δ) or 25 μ g ($\circ$) of G-25 protein fraction. Different amounts of TyrRS were added as indicated in the figure. After transcription, the pre-tRNA was estimated by densitometry as described in Fig. 2 legend. One arbitrary unit corresponds to the amount of pre-tRNA synthesized at low extract concentration without TyrRS.

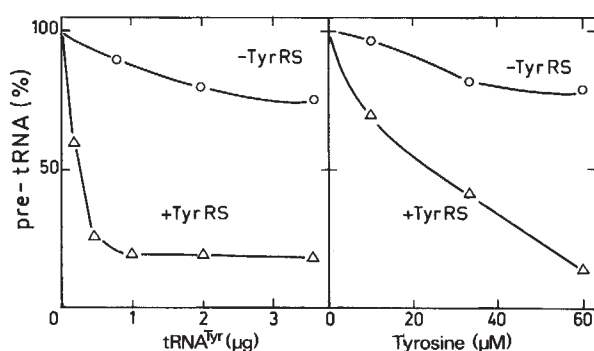


Fig. 4 Effect of tRNA^{Tyr} and tyrosine on *in vitro* transcription of tRNA^{Tyr} gene. Transcription mixtures, described in Fig. 1 legend contained either no TyrRS ($\circ$) or 0.6 μ g TyrRS (Δ) and varying concentrations of tRNA^{Tyr} (left) or tyrosine (right), as indicated. To obtain a comparable tRNA signal, the amount of yeast extract was 4 μ g in reactions with TyrRS and 16 μ g in reactions without TyrRS.

separate protein fractions, one could reproduce the stimulation of tRNA^{Tyr} synthesis by tyrosyl-tRNA synthetase.

These *in vitro* results suggest a role for aminoacyl-tRNA synthetases in the regulation of tRNA synthesis. Our observations indicate that tyrosyl-tRNA synthetase could stimulate transcription when the pools of its substrates, tRNA^{Tyr} and tyrosine, are depleted. The fact that purified tRNA^{Tyr} does not significantly inhibit the synthesis of pre-tRNA^{Tyr} in the crude extract suggests that endogenous TyrRS present in the extract is not predominantly involved in transcription (and also that the transcription factor(s) do not have a high affinity for tRNA). Indeed, about 90% of the endogenous TyrRS activity of the extract could be specifically removed by immunoadsorption, with specific antibodies to TyrRS coupled to protein A-Sepharose, without producing much reduction in the efficiency of transcription (result not shown). Therefore, TyrRS appears to be a modulator rather than a compulsory component of the transcription system. It would be interesting to know if the transcription of other tRNA genes is stimulated by the cognate tRNA synthetase. In preliminary experiments we found no stimulation of tRNA^{Ser} synthesis by seryl-tRNA synthetase from yeast or sheep liver, which both aminoacylate yeast tRNA^{Ser} (J. P. Waller, personal communication).

We thank S. Blanquet and J. P. Waller (Ecole Polytechnique, Palaiseau) for many discussions, helpful suggestions and gifts of Ap₄A (S.B.), sheep liver seryl-tRNA synthetase and phenylalanyl-tRNA synthetase (J.P.W.). We also thank M. Olson for plasmids containing the SUP4 and SUP5 tRNA genes, and R. Koski for making his results available to us before publication. W.S. was supported by a grant from the ADRC (Villejuif).

Received 21 April; accepted 29 June 1983.

- Hofstetter, H., Kressmann, A. & Birnstiel, M. L. *Cell* **24**, 573–585 (1981).
- Galli, G., Hofstetter, H. & Birnstiel, M. L. *Nature* **294**, 626–631 (1981).
- Ciliberto, G., Castagnoli, L., Melton, D. A. & Cortese, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1195–1199 (1982).
- Shastri, B. S., Ng, S. Y. & Roeder, R. G. *J. biol. Chem.* **257**, 12975–12986 (1982).
- Segal, J., Matsui, T. & Roeder, R. G. *J. biol. Chem.* **255**, 11986–11991 (1980).
- Hall, B. D., Clarkson, S. G. & Tochini-Valentini, G. *Cell* **29**, 3–5 (1982).
- Engelke, D. R., Ng, S. Y., Shastri, B. S. & Roeder, R. G. *Cell* **19**, 717–728 (1980).
- Pelham, H. R. B. & Brown, D. D. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4170–4174 (1980).
- Sakonju, S. & Brown, D. D. *Cell* **31**, 395–405 (1982).
- Klekamp, M. S. & Weil, P. A. *J. biol. Chem.* **257**, 8432–8441 (1982).
- Koski, R. A., Worthington, M., Allison, D. S. & Hall, B. D. *Nucleic Acids Res.* **10**, 8127–8143 (1982).
- Faulhammer, H. G. & Cramer, F. *Eur. J. Biochem.* **75**, 561–570 (1977).
- Rapaport, E. & Zamecnik, P. C., *Proc. natn. Acad. Sci. U.S.A.* **73**, 3984–3988 (1976).
- Plateau, P., Mayaux, J. F. & Blanquet, S. *Biochemistry* **20**, 4654–4662 (1981).
- Putney, S. D. & Schimmel, P. *Nature* **291**, 632–635 (1981).
- Olson, M. V. *et al. Nature* **291**, (1981).
- Goddman, H. M., Olson, M. V. & Hall, B. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5453–5457 (1977).

The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour virus

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Glucocorticoids are known to induce the transcription of integrated proviral mouse mammary tumour virus (MMTV) genes in a variety of cell lines derived from mouse mammary tumours¹. Chimaeric genes in which selectable markers are linked to the long terminal repeat (LTR) region of MMTV can be induced by the synthetic glucocorticoid dexamethasone after introduction into mouse fibroblasts^{2–5}. This suggests that the regulatory elements required for hormonal induction are located within the cloned LTR fragments. The idea is supported by the observation that glucocorticoid receptors bind to certain cloned fragments of MMTV DNA *in vitro*^{6–9}. Using filter binding studies and monoclonal antibodies to the glucocorticoid receptor¹⁰ we have now delimited the receptor binding region to a DNA segment of 152 base pairs (bp) that has been shown to be relevant for hormonal induction¹¹. In nuclease protection experiments we have identified partially homologous receptor binding sequences located in this region, all of which share the hexanucleotide 5'-TGTTCT-3'.

We have shown previously that partially purified rat liver glucocorticoid receptor binds to DNA fragments of proviral MMTV containing the right half of the LTR region⁹. These experiments were performed with two different forms of the receptor: a 90,000-molecular weight (M_r) form that was only 50% pure¹², and a 40,000- M_r form that probably represents a proteolytic degradation product, but was 90% pure¹³. Although both preparations showed similar preference for the MMTV-LTR region, there is no conclusive evidence that the receptor and not a contaminating protein is responsible for the observed DNA binding specificity. To test this possibility, we have prepared monoclonal antibodies to the glucocorticoid receptor that do not interfere with receptor binding to DNA¹⁰. Using these antibodies, it is possible to study the binding of receptor to DNA fragments in crude liver cytosol, thus preventing potential damage of the receptor during the purification. For these studies we used a set of chimaeric plasmids that were constructed by linking the thymidine kinase (*tk*) gene of herpes simplex virus to fragments of MMTV DNA exhibiting deletions of different length in the LTR region¹¹. Gene transfer experiments have shown that the transcription of the LTR promoter is markedly enhanced by glucocorticoids, provided that the construction

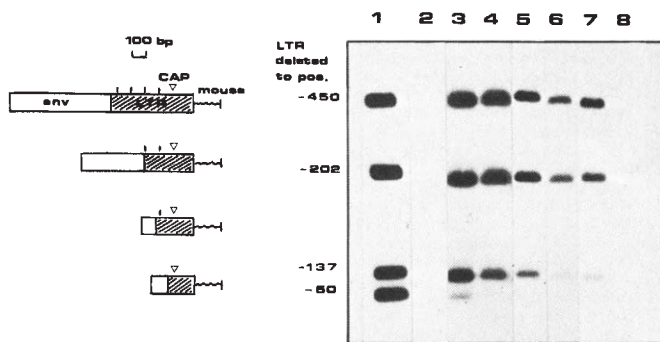


Fig. 1 Delimitation of the receptor binding sites with LTR deletion mutants. Four chimaeric plasmids in which the left part of the LTR region had been deleted up to the indicated positions upstream of the cap site were digested with *Eco*RI, end-labelled with 32 P (ref. 9), and aliquots (10 ng DNA, 150,000 d.p.m.) were used for *in vitro* binding studies. A schematic representation of the fragments used is shown on the left. The right part of the figure represents an autoradiogram of DNA fragments separated by agarose gel electrophoresis. Lane 1 shows the input DNA mixture (a 4.3-kilobase fragment containing pBR322 and *tk* sequences is not shown). Lanes 2–6 show results of nitrocellulose filter binding studies with partially purified receptor preparations⁹. Lane 2 shows the DNA retained on the filter in the absence of receptor. Lanes 3 and 4 show the fragments retained on the filter at 60 mM KCl by the 40,000- M_r form (8 ng) and the 90,000- M_r form (5 ng) of the partially purified receptor respectively. Lanes 5 and 6 show the fragments retained by the 90,000- M_r form of the receptor (18 ng) at either 120 mM KCl (lane 5) or at 60 mM KCl in the presence of 50 ng native calf thymus DNA (lane 6). Lanes 7 and 8 show the fragments precipitated by immobilized monoclonal antibodies following incubation with rat liver cytosol (5 μ l) in the absence (lane 7) or in the presence (lane 8) of 10 mM Na_2MoO_4 . The rat liver cytosol had been previously labelled for 2 h at 0°C with 50 nM ^3H -triamcinolone acetone (specific activity 5 Ci mmol^{-1}). After incubation of the cytosol with DNA, monoclonal antibodies coated to 10 μ l of inactivated *Staphylococcus aureus* (Pansorbin, Calbiochem-Behring) were added and incubation was continued at 4°C for 60 min. Coating was carried out as described previously¹⁴, by incubating 10 μ l of Pansorbin with 200 μ l of supernatant culture from the hybridoma clone 1GR49¹⁰. After centrifugation and washing of the immunoprecipitates, the bound DNA fragments were solubilized with SDS and extracted with phenol, before precipitation with ethanol and electrophoresis in 1% agarose gel.

contains at least 202 bp of the LTR region upstream of the transcription initiation site¹¹. If this region is further deleted to position -50 upstream of the 'cap' site, the hormonal inducibility decreases dramatically¹¹. We have used these chimaeric plasmids in filter binding studies with partially purified preparations of glucocorticoid receptor, and in immunoprecipitation experiments with crude cytosol¹⁴.

Figure 1 shows an example of the results obtained. Both forms of the receptor bind preferentially to fragments containing 450 or 202 bp upstream of the LTR cap site (lanes 3, 4), and to a lesser extent to fragments containing only 137 bp upstream of the cap site. In fragments in which the LTR sequence upstream of -50 was deleted, the preferential binding of the receptor was virtually lost. These results were more evident at higher salt concentrations (Fig. 1, lane 5) or in the presence of calf thymus DNA (lane 6). In addition, the experiments with crude cytosol and monoclonal antibodies clearly demonstrate a preferential binding of the receptor to fragments containing at least 202 bp upstream of the initiation site (Fig. 1, lane 7). Controls without added cytosol or without antibody (data not shown) gave negative results, similar to those obtained when the cytosol was preincubated with 10 mM Na_2MoO_4 (Fig. 1, lane 8), a treatment known to prevent activation of the glucocorticoid receptor¹⁵.

Figure 2 summarizes our results with both forms of the partially purified receptor or with crude cytosol and with eight

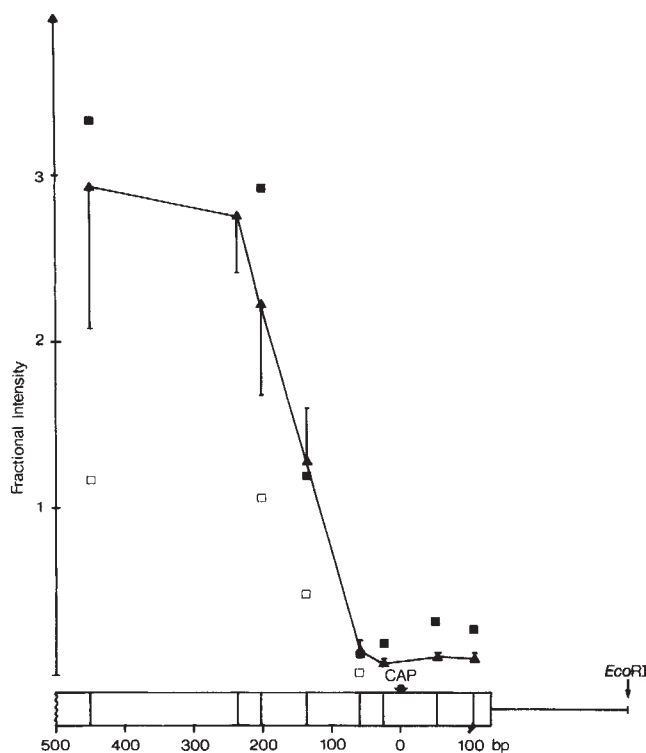


Fig. 2 Summary of the binding experiments with LTR deletion mutants. Two independent experiments similar to that shown in Fig. 1 were evaluated by scanning the autoradiograms with a densitometer. The results obtained with the 90,000- M_r form ($\blacktriangle$) and the 40,000- M_r form ($\blacksquare$) of the partially purified receptor as well as those obtained with crude cytosol and monoclonal antibodies ($\square$) are shown. The average and, when pertinent, the range are indicated. The abscissa shows the extent of the individual deletions as given by the number of base pairs upstream of the RNA initiation site (cap) present in the individual probes. The ordinate represents the fractional intensities of the individual bands in lanes of the autoradiograms corresponding to incubations with receptor, divided by the fractional intensity of the same bands in the input DNA.

different LTR deletions. Several conclusions can be drawn from these experiments. (1) The receptor is the factor responsible for selective DNA binding, since the results with monoclonal antibodies confirm those obtained with partially purified receptor preparations. (2) Other components of the cytosol do not improve the selectivity of DNA recognition, as the magnitude of preferential binding was similar with crude cytosol and with purified receptor. (3) The receptor binding sequences in the MMTV LTR region are located between nucleotides -50 and -202 upstream of the RNA initiation site, in the segment of the DNA known to be required for hormonal regulation¹¹. (4) The finding that the deletion containing only 137 bp upstream of the cap site still binds the receptor but with lower efficiency suggests that there may be at least two binding sites for the receptor in the LTR region.

To define directly the sequence of nucleotides recognized by the glucocorticoid receptor, we used the 'footprinting' technique developed by Galas and Schmitz¹⁶. The restriction fragments used for these studies are shown in Fig. 3. The deletion mutant p13-13 (ref. 11), which contains only 237 bp of the LTR region upstream of the RNA initiation site, was digested with *Bam*HI and end-labelled with 32 P. Second digestion with *Rsa*I produced two fragments of 210 and 438 bp. In filter binding studies the 90,000- M_r form of the glucocorticoid receptor shows a 50-fold preference for the 438-bp fragment, which contains the receptor recognition site(s), over the 210-bp frag-

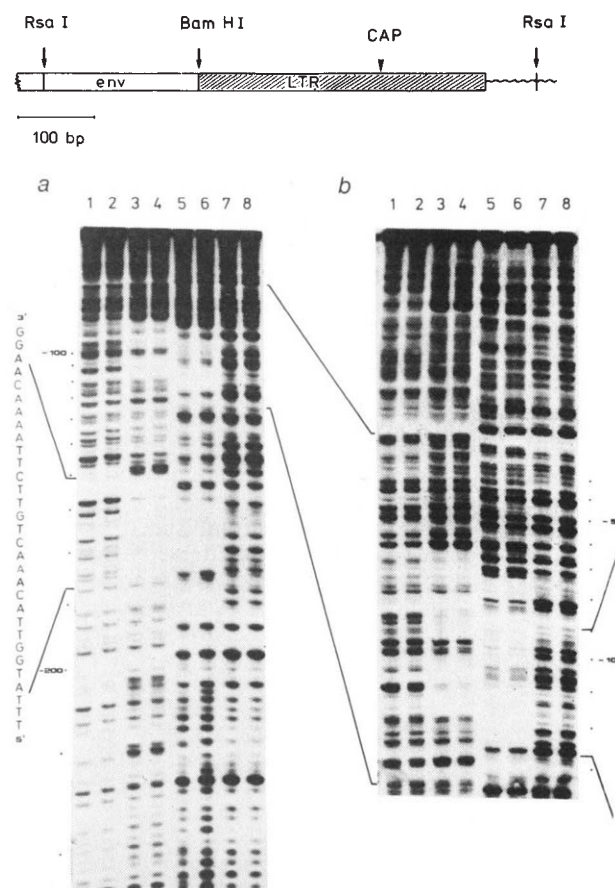


Fig. 3 DNase I protection experiments. Analysis in 8% (a) or 6.5% (b) polyacrylamide urea gels²⁰. The sequence corresponding to the DNase I protected regions is indicated. Lanes 1, 2, control DNase I ladders from 5' end-labelled DNA without added receptor. Lanes 3, 4, the same DNA incubated with the partially purified 90,000-M_r form of the receptor, 2 and 4 $\mu\text{g ml}^{-1}$, respectively. Lanes 5, 6, 3' end-labelled DNA with the same amount of receptor as lanes 3 and 4. Lanes 7, 8, control DNase I ladder from 3' end-labelled DNA without added receptor. Numbers refer to the distance from the RNA initiation site.

Methods: The 438-bp *Bam*HI-*Rsa*I fragment (top) was labelled in the *Bam*HI site either at the 3' end (coding strand) with [α -³²P]dGTP and the large fragment of DNA polymerase, or at the 5' end (messenger strand) with [γ -³²P]ATP and the T4 polynucleotide kinase²¹. Aliquots (100,000 d.p.m.) were incubated at 25°C for 45 min with or without receptor in 100–200- μl assays containing 10 mM Tris-HCl pH 8.0, 100 mM KCl, 1 mM MgCl₂, 1 mM Na₂EDTA, 1 mM 2-mercaptoethanol, 1 mg ml⁻¹ bovine serum albumin and 10% glycerol. DNase digestion was performed as described elsewhere¹⁶. The temperature was shifted to 20°C and 5 min later 20 μl of a freshly prepared mixture of calf thymus DNA (0.25 $\mu\text{g } \mu\text{l}^{-1}$) and DNase I (Worthington, 2.5 ng ml⁻¹) in 30 mM MgCl₂ was added. After 2 min, digestion was stopped with 3 μl 0.3 M Na₂EDTA followed by phenol extraction. The DNA was precipitated with ethanol, rinsed and analysed. Base-specific sequencing reaction, for G and C+T, were performed with the same fragments and applied in parallel (not shown).

ment. On addition of the 90,000-M_r form of the receptor to the 438-bp fragment two DNA regions are protected against nuclease digestion; one region is located between nucleotides -72 and -124 and the other between nucleotides -163 and -192 (Fig. 3, lanes 3–6). The lengths of the protected regions are similar in both strands of the DNA fragment (compare Fig. 3, lanes 3, 4 with lanes 5, 6). The DNase I protection pattern observed in Fig. 3 could be prevented if, before incubation with DNA, the partially purified receptor preparation is deprived of receptor by treatment with immobilized monoclonal antibody (data not shown). Outside the DNase I protected segments certain sites are more readily cut than in the controls. This

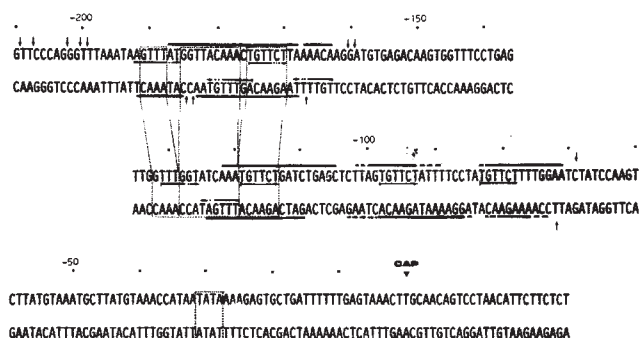


Fig. 4 The DNA sequences protected by the glucocorticoid receptor in the LTR region. Summary of the data shown in Fig. 3. Fat lines indicate DNase I protected regions, dashed ends have been used when the protection border cannot be determined accurately. Solid lines with arrows in between the strands indicate palindromic structures and direct repeats. Vertical arrows indicate sites exhibiting receptor-induced enhancement of DNase I cutting. A homology between the upstream protected region and the 5' end part of the downstream protection system is boxed with dotted lines. The two areas match, except for insertion/deletion of three nucleotides, to 89%. The TATA box and the RNA initiation site are indicated. Numbers refer to the distance from the cap site.

phenomenon was also observed with the 210-bp *Rsa*I-*Bam*HI fragment (Fig. 3, top) which does not show specific receptor binding, and is probably unrelated to the interaction of the receptor with the DNase I protected segments.

The nucleotide sequence we determined in the LTR region essentially confirms previous results¹⁷ and is shown in Fig. 4. The sequence between -113 and -132 is homologous to that between -170 and -191, and both sequences overlap with the strongly protected regions. The other protected regions are not significantly homologous, but the sequence 5'-TGTCT-3' is common to all four subregions. A search for homologies between the protected sequences reported here and nucleotide sequences around the promoters of other known glucocorticoid regulated genes has yielded interesting results. The major DNaseI protected regions (Fig. 4) show strong homology to a region located between -247 and -263 in the human metallothionein IIA gene, known to be induced by glucocorticoids²⁰. Between nucleotides -125 and -107 there is a 19-bp region showing 63% homology to a sequence which is postulated to be recognized by the progesterone receptor¹⁸. The sequence between nucleotides -109 and -100 is 70% homologous to a similarly located sequence in the rat liver gene for tryptophan oxygenase, another glucocorticoid inducible gene¹⁹. The significance of these homologies for hormonal control remains to be established.

Our data support the model for regulation of gene expression, where gene regulators are proteins that as complexes with small molecules (here steroid hormone receptor complexes) interact with defined nucleotide sequences close to the regulated promoters. The complexity of the animal genome, however, and the variable inducibility of the same genes in different target cells demand additional mechanisms that determine the availability of regulable genes. This level of gene control, which may involve covalent modifications of nucleotides and changes in the chromatin structure, has not yet been considered in our studies, although it probably represents a very important aspect of cell differentiation.

Finally, in addition to the receptor binding sites in the LTR region reported here, we have found other sites within the MMTV genome and in the flanking mouse DNA that exhibit affinity for the glucocorticoid receptor⁹. The functional significance of these interactions for the physiological regulation of MMTV expression is unknown.

We thank Bernd Groner and Nancy Hynes for providing the DNA probes and making their results available to us before publication. This work was supported by grants from the DFG and FCI.

Received 21 March; accepted 30 June 1983.

- Ringold, G. M. *Biochim. biophys. Acta* **560**, 487–508 (1979).
- Buetti, E. & Diggelmann, H. *Cell* **23**, 335–345 (1981).
- Huang, A. L., Ostrowski, M. C., Berard, D. & Hager, G. L. *Cell* **21**, 245–255 (1981).
- Hynes, N. E., Kennedy, N., Rahmsdorf, U. & Groner, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2038–2042 (1981).
- Lee, F., Mulligan, R., Berg, P. & Ringold, G. *Nature* **294**, 228–232 (1981).
- Payvar, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6628–6632 (1981).
- Govindan, M. V., Spiess, D. & Majors, J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5157–5161 (1982).
- Pfahl, M. *Cell* **31**, 475–482 (1982).
- Geisse, S. *et al. EMBO J.* **1**, 1613–1619 (1982).
- Westphal, H. M., Moldenhauer, G. & Beato, M. *EMBO J.* **1**, 1467–1471 (1982).
- Hynes, N. E. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Wrange, Ö., Carlstedt-Duke, J. C. & Gustafsson, J. A. *J. biol. Chem.* **254**, 9284–9290 (1979).
- Westphal, H. M. & Beato, M. *Eur. J. Biochem.* **106**, 395–403 (1980).
- Scheller, A., Covey, L., Barnett, B. & Prives, C. *Cell* **29**, 375–383 (1982).
- Leach, K. L., Dahmer, M. K., Hammond, N. D., Sando, J. J. & Pratt, W. B. *J. biol. Chem.* **254**, 11884–11890 (1979).
- Galas, D. & Schmitz, A. *Nucleic Acids Res.* **5**, 3157–3170 (1978).
- Fasel, N., Pearson, K., Buetti, D. & Diggelmann, H. *EMBO J.* **1**, 3–7 (1982).
- Mulvihill, E. R., Le Penec, J. P. & Chambon, P. *Cell* **28**, 621–632 (1982).
- Schmid, W. *et al. EMBO J.* **1**, 1287–1293 (1982).
- Karin, M. & Richards, R. I. *Nature* **299**, 797–802 (1982).
- Maxam, A. & Gilbert, W. *Meih. Enzym.* **65**, 499–560 (1980).

Dependence of DNA helix flexibility on base composition

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We have used triplet anisotropy decay techniques to study the flexibility of synthetic DNA fragments with different base pair compositions. We have found major differences in the torsional and bending stiffness of poly(dG)·poly(dC), poly(dA)·poly(dT) and poly(dA-dC)·poly(dT-dG). Poly(dG)·poly(dC) has a torsional modulus more than 40 times larger than poly(dA-dC)·poly(dT-dG), and approximately 20 times larger than poly(dA)·poly(dT). These differences imply that the torsional stiffness of DNA can vary greatly with base composition. The Young's modulus (bending stiffness) we have measured for poly(dG)·poly(dC) is at least twice that of poly(dA-dC)·poly(dT-dG) or random sequence DNA, and is at least threefold greater than that of poly(dA)·poly(dT). This implies that the bending stiffness of DNA is also strongly dependent on base composition. In light of this dramatic base composition dependence, we suggest here that such stiffness variation may lead to local variations in the stability of chromatin or other protein complexes that require bending or twisting of the DNA helix.

We have shown previously that the triplet anisotropy decay technique can be a powerful tool for studying DNA flexibility^{1,2}. In that technique the brownian motion of DNA helices is monitored by methylene blue, a dye probe that we^{1,2} and others³ have shown to bind by intercalating into the helix. Based on measurements with random sequence DNA, we have concluded that methylene blue serves as an accurate probe of DNA motions^{1,2}. Structural studies have shown that the synthetic DNAs poly(dA)·poly(dT), poly(dA-dC)·poly(dT-dG) and poly(dG)·poly(dC) form B-type helices⁴. It was important to confirm that methylene blue intercalates tightly into these synthetic DNAs. In Table 1, we have summarized the properties of methylene blue-DNA complexes in conditions identical to those used for anisotropy decay measurements. The orientation angle ($72^\circ \pm 2^\circ$) of the methylene blue transition dipole with respect to the helix axis has been measured by linear dichroism, and is in good agreement with values measured previously^{1,2}. The measured orientation of the base pair transition moments ($72^\circ \pm 2^\circ$) is also as expected for B-DNA helices⁵.

As seen in Table 1, the binding constant of methylene blue, and the quenching of methylene blue fluorescence increase with G+C content of the synthetic DNA. A similar increase in

quenching of triplet yield is also seen (not shown). Such base composition-dependent effects are well known for intercalating dyes such as methylene blue³, and have been attributed to stacking of the dye heterocycle next to a G rather than an A residue: G is more polarizable, giving rise to a more favourable stacking interaction and a larger perturbation of dye optical properties⁶. Based upon the similarity of these dye-nucleic acid complexes we conclude that methylene blue binds by intercalation to synthetic DNA helices to form a tight complex which is very similar to that formed with other random sequence DNAs.

In Fig. 1 we show anisotropy decay data for three synthetic DNA fragments, over a time range from 20 ns to 20 μ s. The fragments are all 400 ± 60 base pairs (bp) long and have been prepared to be free from nicks, hairpins or other structures susceptible to S₁ nuclease digestion (see legend to Fig. 1). Each has been tagged with one methylene blue per 70 bp. Halving the density of bound methylene blue had no effect on the anisotropy decay data (not shown).

The rate of anisotropy decay is a direct measure of molecular motion; rapid motions give rise to rapid decay^{1,2}. Because in Fig. 1 we display data for DNA fragments with identical contour length, segmental motion (flexibility) of different helices can be compared by direct inspection. In earlier work with defined length DNA fragments we confirmed that at times greater than 2 μ s, anisotropy decay monitors motion of the long helix axis¹. As seen in Fig. 1, this long time decay rate varies substantially as a function of helix composition: poly(dA)·poly(dT) > poly(dA-dC)·poly(dT-dG) > poly(dG)·poly(dC). In general, the long axis motions being monitored can be decomposed into two parts: the overall tumbling of the fragment and the internal 'warping' motions of the long axis¹. These three DNA fragments have identical contour lengths, so their long axis motions would be identical if the fragments were rigid. Thus, the substantial variation measured in these long-time components suggests in a direct way that the internal bending motions of these helices differ greatly, poly(dA)·poly(dT) being much more flexible with respect to bending than poly(dG)·poly(dC).

We^{1,2} and others⁷ have shown that in the time range from 2 ns to 200 ns DNA anisotropy decay is dominated by torsional motion (the overall 'speedometer cable' motion of the helix and segmental 'twisting' motions of the base pairs). Again, because the dimensions of these DNA fragments are the same, the differences seen in Fig. 1 must reflect differences in the internal flexibility of the fragments. It is clear from the data that poly(dG)·poly(dC) shows the least torsional flexibility (slowest anisotropy decay). In accord with the order of bending flexibility, poly(dA)·poly(dT) is much more flexible than poly(dG)·poly(dC); however, it appears to be stiffer with respect to twisting than poly(dA-dC)·poly(dT-dG). To quantify these differences we have fitted our anisotropy decay data to

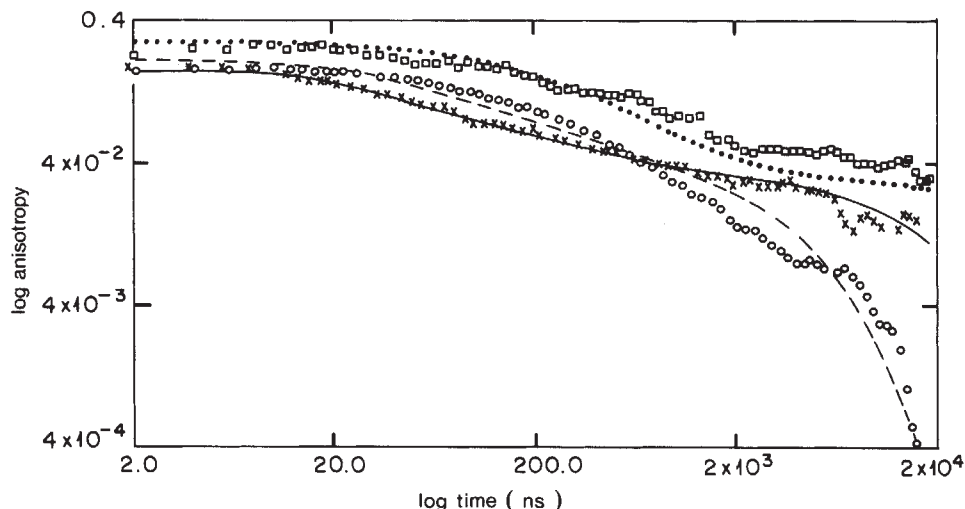
Table 1 Properties of methylene blue complexes with synthetic DNA helices

Sample	K (M ⁻¹)	I/I_0	θ_{MB}	θ_{DNA}
poly (dG)·poly (dC)	2×10^6	0.025	73°	74°
poly(dA-dC)·poly(dT-dG)	1×10^6	0.10	70°	72°
Chicken DNA	5×10^5	0.17	72°	71°
poly (dA)·poly (dT)	1×10^5	0.59	71°	72°

All measurements were made in TE buffer at 5 °C. DNA samples were prepared as described in the legend to Fig. 1. The apparent binding constant K was determined from the quenching of methylene blue fluorescence, using standard techniques¹⁸. The ratio I/I_0 is the ratio of methylene blue fluorescence intensity in the bound and unbound states. Fluorescence measurements were made on a Perkin-Elmer fluorimeter: λ_{ex} , 665 nm; λ_{em} , 685 nm. The angle θ_{MB} between the long helix axis and the methylene blue absorption dipole was measured at 665 nm using electric dichroism techniques and a device generously provided by D. M. Crothers, Yale University¹⁹. The angle θ_{DNA} between the DNA base transition moments and the long helix axis was measured at 265 nm as described earlier¹⁹.

Fig. 1 Triplet state anisotropy decay of synthetic DNA fragments. Data are shown for fragments with identical contour length (400 ± 60 bp), that is, every DNA sample had an average length equal to 400 bp and greater than 80% of the DNA in each sample had lengths within 60 bp of that average. $\square$, poly(dG)·poly(dC); $\circ$, poly(dA)·poly(dT); $\times$, poly(dA-dC)·poly(dT-dG). Simulations of the data using equations (1) and (2) and parameters cited in Table 2: $\cdots$, poly(dG)·poly(dC); $---$, poly(dA)·poly(dT); $---$, poly(dA-dC)·poly(dT-dG).

Methods: Measurements were made in TE buffer (10 mM Tris-HCl, 0.01 mM Na_2EDTA , pH 7.4) at 5°C at a methylene blue binding density of 1 per 70 bp. DNA concentration was adjusted to be greater than 30 times the dissociation constant of methylene blue. Tripling the ionic strength or DNA concentration had no effect on the data (not shown). Synthetic, double-stranded DNAs were purchased from Boehringer. Chicken DNA was isolated from adult erythrocytes, following standard procedures¹⁶. DNA for these measurements was digested with nuclease to remove single-strand regions, then fractionated by length, as described elsewhere¹⁷. Briefly, DNA was digested in 50 mM NaOAc, 2 mM ZnCl₂, 0.1 M NaCl at pH 4.9 for 10 min at 37°C with 0.5 units of S_1 nuclease per μg DNA. Digestion was terminated by adding EDTA to 10 mM and cooling. DNA was then treated with 50 $\mu\text{g ml}^{-1}$ protease K at 37°C for 2 h, phenol-extracted twice, then fractionated by length on a 50 cm column of Sepharose 6B. DNA length was measured by gel electrophoresis in native and denaturing conditions, relative to length standards ($\Phi\text{X174 HaeIII}$ digest). For each DNA sample fractionated as described above, double-strand and single-strand lengths were measured to be identical, confirming that the material is free from nicks. In all cases, DNA prepared in this fashion was insensitive to subsequent S_1 nuclease digestion (not shown), confirming that the DNA is free from loops or hairpin domains.



the theory of Allison and Schurr (ref. 8 and J. M. Schurr, personal communication) modified, as suggested by Schurr (personal communication), with an expression for helix bending motion derived by Barkley and Zimm⁹.

Schurr has modelled the DNA helix as a variable number of stacked base pairs interconnected by torsional springs⁸. Consequently, base pair twisting motions are described in terms of a torsional spring constant C . Bending motions are introduced by assuming that the long axis of the helix can experience the wave-like 'warping' motions characteristic of an elastic coil, and are parameterized by the Young's modulus E within the Barkley and Zimm equation for bending motion of the helix⁹.

The anisotropy $r(t)$, as a function of bending and twisting of the helix, is written as

$$r(t) = 0.4[1/4(3 \cos^2 \theta - 1)^2 F_1 + 3(\sin^2 \cos^2 \theta) F_2 C_1 + 3/4 \sin^4 \theta F_3 C_2] \quad (1)$$

Here θ is the angle between helix axis and the transition dipole responsible for methylene blue absorption. C_1 and C_2 are the twisting decay functions, and F_1 , F_2 and F_3 are the bending decay functions defined by Allison and Schurr⁸. The bending functions are

$$F_1 = \exp[-1.5\Delta(t)]; F_2 = (F_1)^{5/6}; F_3 = (F_1)^{2/6} \quad (2)$$

where $\Delta(t)$ is the bending function derived by Barkley and Zimm⁹. Although equations (1) and (2) are complicated, they have only two unknown parameters: the torsional spring constant and Young's modulus. Other parameters in equation (2) are measured independently.

In Fig. 1 we show the best simulations of the 400-bp data using equations (1) and (2). The anisotropy decay data and the simulations to that data converge to a limiting anisotropy near 0.3 at the earliest times. That limiting anisotropy is equal to the value we measure for methylene blue in 98% glycerol, or in rigid plastic matrices (not shown). Such close correspondence suggests that fast, unresolved dye motions are not occurring in these measurements. Table 2 presents values for the torsional spring constant C and Young's modulus E calculated from anisotropy decay measurements of 400-, as well as 260- and

800-bp long DNA fragments. We also present for comparison, a time constant τ for each DNA corresponding to the best fit of the slowest part of the decay curves to an exponential function. For 400-bp long DNA this time constant simply quantifies what is seen by inspection of Fig. 1: the rate of anisotropy decay at long times decreases in the order poly(dA)·poly(dT) > poly(dA-dC)·poly(dT-dG) > poly(dG)·poly(dC).

As seen in Table 2, the Young's modulus calculated from 400-bp long DNA data reflects that same trend; its value for poly(dG)·poly(dC) is approximately three times that of poly(dA)·poly(dT), and approximately twice that of poly(dA-dC)·poly(dT-dG).

Young's modulus can be related to the hydrodynamic persistence length P of a helix by a simple relationship derived by Landau and Lifshitz¹⁰

$$P = EI/kT \quad (3)$$

where I is the surface moment of inertia (which depends only on the dimensions of the helix), k is Boltzmann's constant and T is temperature in kelvin. We have used this expression to calculate the persistence length for each of these fragments (Table 2). To our knowledge, such hydrodynamic measurements have not been reported for synthetic DNA helices, therefore to facilitate comparison we have made anisotropy decay measurements on a 400 ± 60 -bp long random sequence chicken DNA fragment and present the result of fits to those data in Table 2.

As seen, the persistence length and Young's modulus E for chicken DNA (43% GC) and poly(dA-dC)·poly(dT-dG) are identical. The persistence length which we calculate for the 400-bp chicken DNA fragment is 25–50% greater than comparable values measured at 20°C by sedimentation velocity¹¹ or light scattering measurements¹². Considering the approximations involved in the available theory of anisotropy decay, we consider the agreement to be good, suggesting that anisotropy decay measurements provide an accurate measure of DNA bending motions.

Note that the Barkley–Zimm bending theory which we have used is an approximation which holds rigorously only in the

Table 2 Elastic properties of defined length DNA helices

Sample	C (erg)	τ (μ s)	P (bp)	E (dyn cm ⁻²)	Length (bp)
poly(dG)·poly(dC)	4.0×10^{-11}	60.0	400	2.3×10^9	400
poly(dA-dC)·poly(dT-dG)	9.0×10^{-13}	16.0	250	1.4×10^9	400
Chicken DNA	1.0×10^{-12}	15.0	250	1.4×10^9	400
poly(dA)·poly(dT)	2.0×10^{-12}	3.5	150	8.2×10^8	400
poly(dG)·poly(dC)	4.0×10^{-11}	40.0	260	1.5×10^9	260
poly(dA-dC)·poly(dT-dG)	1.0×10^{-12}	6.0	175	1.0×10^9	260
Chicken DNA	1.0×10^{-12}	6.0	175	1.0×10^9	260
poly(dA)·poly(dT)	2.0×10^{-12}	3.0	125	7.3×10^8	260
poly(dG)·poly(dC)	4.0×10^{-11}	100	500	2.9×10^9	900

Parameters are derived from a best fit of equations (1) and (2) to anisotropy decay data. 400 ± 60 , 260 ± 40 and 800 ± 200 bp long DNA fragments were prepared as described in the legend to Fig. 1. C, Torsional spring constant calculated from equations (1) and (2); τ , exponential time constant for motion of the long helix axis estimated from a fit of the slowest part of the anisotropy decay data to a single exponential; P, the apparent persistence length of the fragments; E, Young's modulus, calculated from fits of data to equations (1) and (2). In these measurements, the laser pulse has a finite width and therefore must be convoluted into the initial 20 ns of anisotropy decay simulation. When fitting our data to equations (1) and (2), we have included such a convolution (the pulse has been included as a 20-ns gaussian excitation profile, using 100 points at 0.2-ns intervals).

limit that the contour length of a helix is much longer than its persistence length⁹. That criterion is not met for 400-bp long poly(dG)·poly(dC). As seen in Table 2 the persistence length of 400-bp dG·dC helices is equal to the contour length, implying that the long helix axis is sufficiently stiff that its tumbling motion is nearly that of rigid rod. Measurements with a longer DNA fragment (Table 2) show that the persistence length of poly(dG)·poly(dC) is actually near to 500 bp. Work is now in progress to characterize the length dependence of these bending parameters in more detail.

As seen in Table 2, the torsional modulus of these helix fragments is also dependent on base composition. Best fits of the data to equations (1) and (2) show that, independent of length, the torsional modulus C for poly(dG)·poly(dC) is at least 20 times that of poly(dA)·poly(dT), and 40 times that of poly(dA-dC)·poly(dT-dG) or chicken DNA. There are no measurements of fractionated synthetic DNA fragments in the literature to compare with our results. However, for comparison, the value of the torsional modulus that we obtain for random sequence chicken DNA is in good agreement with that calculated from fluorescence measurements¹³ and from studies of closed circular DNA supertwisting¹⁴, an equilibrium technique which requires no extrinsic dye probe.

Clearly, additional work is required to understand the origins of DNA flexibility at the molecular level. We have, however, shown in a direct way that DNA sequences with altered stiffness do exist. We suggest that such stiffness variation may be important when considering DNA packaging. For instance, DNA in chromatin is bent to a radius of curvature of 45 Å in the nucleosome core particle¹⁵. If DNA is modelled as an elastic coil, the energy U required to bend the helix is simply related to the persistence length P: $U = PLkT/R^2$, where L is the length of helix to be bent to a radius of curvature R (ref. 10).

On the basis of our measured values for the persistence length, we calculate that, in a nucleosome, the energy required to bend 146 bp of poly(dG)·poly(dC) will be nearly twice that to bend random sequence DNA (or poly(dA-dC)·poly(dT-dG)) and nearly three times that for poly(dA)·poly(dT). Such variation in bending energy provides a mechanism by which the stability of a nucleosome complex may vary with DNA sequence. Ignoring variations in the strength of histone-DNA interactions, the work presented here suggests that G+C-rich DNA sequences could be sites of weakened nucleosome structure while A+T-rich regions may be sites where nucleosome formation is favoured energetically.

We thank M. Schurr for his help with anisotropy theory, D. M. Crothers for the use of transient electric dichroism equipment and E. J. Groth for help with computer programming. This work was funded by NIH grants GM 29047-01 (M.H.) and GM 30789-01 (R.A.) and by PHS grant CA 09167-07 (J.L.).

Received 15 April; accepted 13 June 1983.

- Hogan, M., Wang, J., Austin, R. H., Monitto, C. L. & Hershkowitz, S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3518-3522 (1982).
- Wang, J., Hogan, M. & Austin, R. H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5896-5900 (1982).
- Muller, W. & Crothers, D. M. *Eur. J. Biochem.* **54**, 267-277 (1975).
- Leslie, A. G. W. & Arnott, S. *J. molec. Biol.* **143**, 49-72 (1980).
- Hogan, M., Dattagupta, N. & Crothers, D. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 195-199 (1978).
- Crothers, D. M. & Muller, W. *Cancer Chemother. Rep.* **58**, 97-100 (1974).
- Robinson B. H. *et al. J. molec. Biol.* **139**, 19-44 (1980).
- Schurr, J. M. & Allison, S. A. *Biopolymers* **20**, 251-259 (1980).
- Barkley, M. D. & Zimm, B. H. *J. chem. Phys.* **79**, 2991-3007 (1979).
- Landau, L. & Lifshitz, E. M. *Statistical Physics* (Addison-Wesley, Reading, 1958).
- Kovacic, R. T. & VanHolde, K. E. *Biochemistry* **16**, 1490-1498 (1977).
- Gray, H. B. & Hearst, J. E. *J. molec. Biol.* **35**, 111-129 (1968).
- Millar, D. P., Robbins, R. J. & Zewail, A. H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5593-5597 (1980).
- Depew, R. E. & Wang, J. C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4275-4279 (1975).
- Finch, J. T. *et al. Nature* **269**, 29-36 (1977).
- Tatchell, K. & VanHolde, K. E. *Biochemistry* **16**, 5295-5303 (1977).
- Hogan, M. & Jardtzy, O. *Biochemistry* **19**, 2079-2085 (1980).
- LePecq, J. B. & Paoletti, C. *J. molec. Biol.* **27**, 87-106 (1967).
- Hogan, M., Dattagupta, N. & Crothers, D. M. *Biochemistry* **18**, 280-288 (1979).

Errata

IN the letter 'Z-DNA immunoreactivity in rat tissues' by G. Morgenegg *et al.*, *Nature* **303**, 540-543 (1983), line 8 in paragraph 3 should refer to "modified DNAs in either the A or B-forms", not the Z or B-forms as published.

IN the matters arising reply to 'Overlapping spreading centres on East Pacific Rise' by H. Schouten and K. D. Klitgord, *Nature* **303**, 549-550 (1983), the authors should be shown as K. C. Macdonald and P. J. Fox, and P. J. F.'s address is the Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02881, USA.

IN the letter 'On neutron star structure and the millisecond pulsar' by A. K. Harding, *Nature* **303**, 683-685 (1983), the relation 4 lines above equation (3) should read $P_{eq} = 1 \times 10^{-3} (M/M_{\odot})^{-1/2} R_6^{3/2}$ s and equation (3) should read:

$$B_8 < 1.8 R_6^{-5/4} (M/M_{\odot})^{1/4} M_{17}^{1/2}$$

IN the letter 'Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity' by G. N. Rogers *et al.*, *Nature* **304**, 76-78 (1983), in the list of conserved amino acid residues in the second paragraph of page 78, Gln should read Glu.

Corrigendum

IN the letter 'Three mutant insulins in man' by S. Shoelson *et al.*, *Nature* **302**, 540-543 (1983), the fourth author's name was shown incorrectly and should read K. Nanjo.

Heterozygosity and genetic distance of proteins

In a statistical study of the relationship between genetic distance¹ (D) and average heterozygosity (H), Skibinski and Ward^{2,3} observed that D increased with increasing H but $D > 0$ when $H = 0$. Arguing that D should be 0 when H is 0, they concluded that their observation is inconsistent with the neutral mutation hypothesis. This conclusion is not justified for the following reasons.

First, the fact that $D > 0$ when $H = 0$ indicates that gene substitution has occurred in the evolutionary process for the protein loci involved, and this in turn means that mutation occurs occasionally at these loci. In other words, the mutation rate for these loci is not zero, and thus the expectation of H is not really zero, unlike Skibinski and Ward's assumption. In practice, however, H is subject to large stochastic and sampling errors when it is estimated from a relatively small number of individuals⁴. Indeed, when n genes are sampled from a population, the probability (P) that no variant alleles are found at a neutral locus is $(1/n)^{4N\nu}$, where N and ν are the effective population size and mutation rate, respectively⁵. Thus, if $4N\nu = 0.02$ (yielding an average heterozygosity, H , of ~ 0.02) and $n = 100$, $P = 0.91$. The probability that this locus is monomorphic in two independent species is $P^2 = 0.83$. Therefore, the estimate of H can easily become zero even if the expectation of H is not zero. On the other hand, D increases with evolutionary time, and if the time since divergence between two species is long, D can be large even if ν or the expectation of H is small. In other words, the observation of $D > 0$ when $H = 0$ is perfectly compatible with the neutral theory.

Second, Skibinski and Ward³ used a steady-state model on the supposition that average heterozygosities in related species are more or less similar. While no *a priori* information regarding heterozygosity at different points of time are available, this assumption is not guaranteed as many natural populations have probably experienced bottlenecks of population size in the past. The effects of bottlenecks of population size are quite long-lasting, and furthermore in such a case the initial rate of accumulation of genetic distance is much faster due to larger effects of random drift on D than H (ref. 6). This distorts the relationship between D and H , particularly when heterozygosity is low. Actually, under a non-equilibrium infinite allele model, the relationship between D_t (distance at a time t) and H_t (heterozygosity at time t) may be written

$$D_t = 2\nu t - \ln [(1 - H_0)/(1 - H_t)] \quad (1)$$

which would also make D_t non-zero even if H_t is zero.

In addition, the correlation between the heterozygosity and the evolutionary rate of proteins may be due to differences in the mutation rate for different proteins. Examining only those mutants that are neutral, a protein with relatively high mutation rate should have both a higher heterozygosity and a greater genetic distance between species than a protein with a lower mutation rate. More specifically, the ratio of the genetic distances for two proteins with different mutation rates is $\sim \nu_1/\nu_2$ and the ratio of their heterozygosities is nearly $\nu_1(4N_e\nu_2 + 1)/\nu_2(4N_e\nu_1 + 1)$ where ν_1 and ν_2 are the mutation rates for two different proteins and N_e is the effective population size. If there is a 10-fold difference in mutation rates ($\nu_1 = 10\nu_2$), the genetic distance would differ by almost a factor of 10 and the heterozygosity would differ by almost a factor of 10 (assuming $4N_e\nu_1$ is not too large). The cited genetic distances given in Fig. 1 of ref. 3 range from about 0.1 to 1.0 and the heterozygosities from 0.02 to 0.2. Although there is little direct information of appropriate mutation rates, a 10-fold difference among molecules seems possible due to several factors.

Thus we conclude that the careful and elegant analysis of Skibinski and Ward^{2,3} does not provide any evidence against the neutral mutation hypothesis of molecular evolution.

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1. Nei, M. *Am. Nat.* **106**, 283–292 (1972).
2. Skibinski, D. O. F. & Ward, R. D. *Genet. Res.* **38**, 71–92 (1981).
3. Skibinski, D. O. F. & Ward, R. D. *Nature* **298**, 490–492 (1982).
4. Nei, M. & Roychoudhury, A. K. *Genetics* **76**, 379–390 (1974).
5. Kimura, M. *Theor. Populat. Biol.* **2**, 174–208 (1971).
6. Chakraborty, R. & Nei, M. *Evolution* **31**, 347–356 (1977).

SKIBINSKI AND WARD REPLY—Chakraborty and Hedrick disagree with our conclusion¹ that neutral mutation theory cannot completely account for the observed relationship between protein genetic distance and heterozygosity. We are not convinced, however, that their criticisms are justified.

The basis of our argument was that steady-state neutral theory predicts an approximately linear relationship

between heterozygosity (H) and genetic distance (D) with $D = 0$ when $H = 0$ and with the slope set by the values of the parameters' divergence time (t) and effective population size (N_e). However, our observed linear regression of D on H calculated for a sample of 31 different proteins had a significant intercept on the genetic distance axis. Because our method was designed with the aim of controlling for variation in t and N_e among proteins, we argued that, regardless of the values of its parameters, neutral theory could not explain this result.

Chakraborty and Hedrick first point out that, as a result of sampling error, the observed heterozygosity at a neutral locus may be below its expected value while genetic distance for the locus may be high. Such situations are common in practice, for example when two related species are fixed for different alleles. In our study, however, both H and D were estimated for each protein using a minimum of 30 loci from 30 pairs of species (most sample sizes are much greater). The estimates will therefore be much closer to the expected values than in the single locus example of Chakraborty and Hedrick. Moreover, if sampling variation in H (estimated from the standard error of heterozygosity for each protein) is taken into account in our analysis, the regression constant is reduced by only a negligible amount (from 0.16 to 0.15). Thus the argument of Chakraborty and Hedrick exaggerates the effect of sampling in relation to our study.

It is true that D for an individual protein can be high if the time since divergence is great even if neutral mutation rate (ν) and thus the expectation of H is low. However, for the same time period, relatively larger D values would accumulate, according to neutral theory, for proteins with higher ν and H . The true relationship between H and D for a sample of proteins with different ν is then expected, according to neutral theory, to be approximately linear and to pass through the origin. This is the crux of our argument, not the distances accumulated by individual proteins considered in isolation.

Second, Chakraborty and Hedrick propose a non-equilibrium model which allows for the possibility that heterozygosity changes with time and which they say is consistent with our findings. Although bottlenecks may affect locus heterozygosity within a population or species, the protein heterozygosity values in our study were averages for many loci and many species from different vertebrate groups, and there are no *a priori* reasons to believe that these global average heterozygosity values are very different now from those in the past. Furthermore, we do not see how this non-equilibrium model can account for the non-zero intercept on the genetic distance axis. Deviations from equilibrium could arise either from a

global change in effective population size (N_e) (which, like divergence time, is assumed to be similar for all proteins) or from changes over time in neutral mutation rates of individual proteins. Under the infinite-allele model of neutral alleles, the expected heterozygosity after t generations is given by:

$$H_t = H_\infty + (H_0 - H_\infty) e^{-t/(2N_e(1-H_\infty))} \quad (1)$$

where H_∞ is the steady-state heterozygosity and H_0 the initial heterozygosity. It is easy to show by numerical analysis of this equation and equation (1) of Chakraborty and Hedrick that at steady state a change in N_e will affect the slope of the relationship between D and H but will not cause a positive intercept of the D axis. However, it is true that changes in ν with time could result in intercepts of either the D or H axes. For example, a positive D intercept would be obtained if the low heterozygosity proteins originally had much higher global heterozygosity and, in moving to the present low values as a result of a reduction in neutral mutation rate, have accumulated more genetic distance than would have been predicted from the steady-state model on the basis of their present heterozygosity values. Yet such a *post-hoc* hypothesis is arbitrary and seems implausible. After all, one of the basic tenets of neutral theory is the assumption of constancy of neutral mutation rates for a given protein over long periods of time².

Finally, we agree that the positive correlation between heterozygosity and genetic distance is precisely that expected under neutral theory assuming differences in neutral mutation rate among proteins. We have stated this elsewhere³. It was not, however, this aspect of the results which we wished to draw attention to as being difficult to reconcile with present neutral theory, rather the non-zero regression intercept and relatively high genetic distance of low heterozygosity proteins.

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Measurements on a shock wave generated by a solar flare

MAXWELL AND DRYER¹ have criticized our radio-scattering observations² of the 18 August 1979 shock wave, on two accounts.

First, for the purpose of estimating the shock velocity using the Rankine-Hugoniot relations, Maxwell and Dryer argue that the value of post-shock velocity used should have been $1,250 \text{ km s}^{-1}$ instead of the $2,600 \text{ km s}^{-1}$ that we used. This leads to an inferred shock speed of $2,300 \text{ km s}^{-1}$ instead of $3,500 \text{ km s}^{-1}$. While their arguments might be valid if the solar wind profile were obtained from point measurements, they do not apply to our path-integrated radio-scattering measurements. For the wind velocity deduced from radio-scattering measurements to represent the post-shock *in situ* velocity, a significant fraction of the radio line-of-sight path must be immersed in the post-shock gas. Thus, to estimate accurately the post-shock speed from the radio-scattering measurements, one must choose the maximum of the radio-scattering deduced velocity-time curve. Velocity points on the rising edge will systematically underestimate the true post-shock gas velocity, and, therefore, underestimate the inferred shock speed. Indeed, for a shock speed of $3,500 \text{ km s}^{-1}$, a significant portion of the line-of-sight path is filled by post-shock material after 20 min, the observed rise time of the radio-scattering deduced wind profile (see Fig. 3c of ref. 2). Thus, $3,500 \text{ km s}^{-1}$ remains the best estimate of the shock speed at $13.1R_\odot$, within the limitations of the radio method (such as, not knowing the shock normal).

Second, Maxwell and Dryer also point out that the shock velocity based on the transit time from the flare region to $13.1R_\odot$ should have been $2,350 \text{ km s}^{-1}$ instead of $3,509 \text{ km s}^{-1}$ (ref. 2). The shock velocity deduced² from the transit time was only a secondary estimate, and did not affect the conclusions of ref. 2. Moreover, a corrected estimate of $2,800 \text{ km s}^{-1}$ had already been published by Cane *et al.*³, who presented the first determination of the heliocentric dependence of the shock velocity in the heliocentric distance range 0.05–0.4 AU. These results were based on a combined analysis of the ISEE 3 interplanetary Type II burst drift rate and scintillation measurements of both the Pioneer 11 and Voyager 1 (ref. 2) radio signals.

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MAXWELL AND DRYER REPLY—We would like to point out that one cannot estimate an *in situ* shock velocity by using a plasma velocity other than the immediate post-shock value. Our concern is simply with the radio technique's ability to specify the post-shock velocity immediately behind the shock in the same sense that is presently done by *in situ* spacecraft.

Woo and Armstrong suggest above that "a significant fraction of the radio line-of-sight must be immersed in the post-shock gas". We believe, however, that their peak plasma velocity (Fig. 3c in ref. 1) represents some 'mean' value of the solar wind velocity in the disturbed plasma well behind the shock and not the immediate post-shock plasma velocity. Woo and Armstrong refer to the fact that "velocity points on the rising edge (of the plasma velocity-time curve) will systematically underestimate the true post-shock gas velocity". We point out that our estimate² of $v_2 = 1,250 \text{ km s}^{-1}$ makes adequate compensation in keeping with their point. Hence, if measurements by the radio technique involve uncertainties for such highly spatial- and time-dependent flow, then the uncertainty in the actual plasma velocity immediately behind the shock ought to include our value of $2,300 \text{ km s}^{-1}$ as well as their value of $3,500 \text{ km s}^{-1}$. Thus, we suggest that the latter figure is not necessarily the 'best estimate'. Additional studies of this and other similar events by Woo, Armstrong, and other radio astronomers familiar with the radio method are needed.

Referring to their second point, we draw attention to Fig. 2 of ref. 3; from the points out to $75 R_\odot$, we deduce that the data are best fitted by a straight line that gives a constant shock velocity of $\sim 2,300 \text{ km s}^{-1}$ rather than the $2,800 \text{ km s}^{-1}$ value that is now asserted by Woo and Armstrong. We are, of course, encouraged to notice that the $2,300 \text{ km s}^{-1}$ value for the velocity agreed so closely with the estimate given in our paper².

Finally, a discussion such as this underlines the necessity for combining data sets and analyses of additional events before further progress can be made in this exciting topic of solar-generated travelling interplanetary phenomena.

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1. Skibinski, D. O. F. & Ward, R. D. *Nature* **298**, 490–492 (1982).

2. Kimura, M. & Ohta, T. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2848–2852 (1974).

3. Skibinski, D. O. F. & Ward, R. D. *Genet. Res.* **38**, 71–92 (1981).

1. Maxwell, A. & Dryer, M. *Nature* **300**, 237–239 (1982).

2. Woo, R. & Armstrong, J. W. *Nature* **292**, 608–610 (1981).

3. Cane, H. V., Stone, R. G. & Woo, R. *Geophys. Res. Lett.* **9**, 897–900 (1982).

1. Woo, R. & Armstrong, J. W. *Nature* **292**, 608–610 (1981).

2. Maxwell, A. & Dryer, M. *Nature* **300**, 237–239 (1982).

3. Cane, H. V., Stone, R. G. & Woo, R. *Geophys. Res. Lett.* **9**, 897–900 (1982).

Role of the renewables

F.J.P. Clarke

Alternative Energy Sources: For the Centralised Generation of Electricity

By R.H. Taylor.

Adam Hilger, Bristol: 1983. Pp. 310. £18, \$36

Renewable Energy: The Power to Choose.

By Daniel Deudney and Christopher Flavin.

W.W. Norton: 1983. Pp. 431. \$18.45 £14.50.

ONCE upon a time people saw energy policies as concerned with ensuring adequate and secure energy supplies at the lowest practicable cost to the nation. Indeed, this is the attitude of most governments today. Of course, there has to be due regard to the environment, the disadvantaged and other social factors, but the traditional thrust of policy is adequate supply and efficient use of energy.

Over the past decade or more, however, there has developed an alternative view that society with its increasing centralization, an undue emphasis on productivity and efficiency, its lavish or even wanton use of depleting resources, is heading in the wrong direction. Energy has become one focus for this concern, and nowhere is it more apparent than in the area of renewable energy. A dichotomy is evident even in the title of one of these books, *Renewable Energy: The Power to Choose*. What it is that the authors want the power to choose becomes clear towards the end of the book — it is the power to choose a new society based on this alternative view, one that will rectify many problems perceived in present society. Therefore, to the authors from the Worldwatch Institute in Washington DC, present energy policies tend to be

myopic, focusing on the maximization of energy output with little reference to the critical values — jobs, equity, the environment, and national security — that are affected by energy investments.

Present policies are too dominated by the powerful interests of the established institutions, and

overcoming this entrenched opposition will be possible only if the beneficiaries of renewable energy — farmers, small businesses, environmentalists, consumers, home owners, and the unemployed — band together and pursue coherent political objectives.

Now I suspect that such a political view is the starting point of many similar books and articles, though they often purport to provide simply an objective analysis. Indeed, that is arguably the aim also of this book, "to draw on the decade's experience with renewable energy and critically assess its potential". The general conclusion is that "renewable energy is... an economic alternative to coal and nuclear power...". And for those of us who do not share the political imperative, this economic aspect is the nub of the issue or, more particularly, what size of renewable resource is cost effective or might become

so. That is a very difficult question to answer for any one nation, let alone for the world, because the economics of renewable sources depend so much on geography and climate, often in a complex manner. But apart from these natural factors, artificial effects such as national regulations and tax measures greatly confuse matters. It is very difficult to disentangle all these factors and to determine what is cost effective in national resource terms. The book gives little help in this crucial matter. While statements about economics are scattered around the text, the critical linking of economics and resource size is weak.

Among the solar technologies, the authors pick out passive heating as having the greatest potential (i.e. architectural design to allow a house to absorb the warmth of the Sun), and that would be the view of most experts; however whether it is correct to generalize that potential in the form that, as "a useful rule of thumb... for a 10% higher initial cost, climate sensitive designs can reduce the fuel

bill by a full 80%" seems doubtful. This is particularly so because the authors fail to make the important distinction between purely energy conservation measures and passive solar heating of buildings.

There are excellent chapters on biomass. Too few people realize the seriousness of deforestation, especially with regard to the problems of starvation and reduced life expectancy among a third or so of the world's population. And equally few recognize the immediate potential of biological wastes as an energy source in both developed and undeveloped countries. The book deals well with both topics and is worth reading for these chapters alone.

Hydropower and wind power come next. Wind power is the most promising of the new electricity-producing renewables, and the book explains that the most economical way to use it is by connection to the electricity grid. However, the subsequent text gives little enlightenment on the role of wind power in a typical grid system. The central point here is that one will choose to have it only if it is cheaper than the most efficient coal or nuclear plant, or other firm source of power in the system; but it will not generally replace this nuclear or other base load plant, but rather the plant in the system that is most expensive to run (usually the least efficient coal or oil stations). And its value is principally the fuel cost it saves. It is therefore misleading to compare, as the book does, wind power availability with nuclear power availability in defence of the wind's intermittent nature; they have different roles in the system.

Renewable Energy is a well-written book, extensively referenced (over 20 % of the pages contain reference material), that argues its case in a popular and generally well-informed manner. If one starts with a view about political or social change, then the book must be judged a success in explaining the concomitant role that renewable energy sources could plausibly play. However, it does not really help the reader to judge the still valuable part that these renewable sources might have, even on a "trends continued" view of the future.

The second book, *Alternative Energy Sources*, written by an official of the Central Electricity Generating Board in the UK, does partly fulfil this latter requirement in respect of the electricity-producing renewables. It tackles each of these technologies in a systematic way, considering history, theory, applications, economics and so on.

Onshore wind power — especially in high windspeed sites — and tidal power comes out as the most strongly placed technologies. But the author is non-committal about exactly what might be achieved. He does explain that the most economic sites are windy hilltops, but many of these seem to be ruled out because they are in areas of outstanding beauty. Lower-windspeed

IMAGE
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REASONS

Power for the future? An experimental wind-turbine located in south-west Wales, UK. The machine can supply up to 200 kW of electricity.

CEGB

lowland sites are available, especially in the east of the UK, but they will require advanced designs of machine the environmental acceptability of which remains to be established. The offshore resource is likely to be very large indeed, but a question-mark must remain over its economics; things are always more expensive to achieve offshore than on land. So, while wind power is tempting enough to persuade the UK Electricity Boards to buy and try out machines, it cannot yet be regarded as proved.

Wave power is probably the least attractive of the major renewable technologies, though this is not what the author admits. The problem is that one has to have huge devices, moored or otherwise fixed in seas that are among the wildest in the world, with power being transmitted through cables on the sea bed to the mainland shore, and then across hundreds of kilometres on land in order to reach the industrial centres where the power could be used. Such features are costly. And even for island use, it is not clear that wave power will be preferable to wind power, because most islands are windy. The author comes to the neutral conclusion that while there may be a potential for costs to be increased or reduced in the future, "wave power remains an interesting insurance technology which . . . needs to be further studied"; this is Establishment code for the fact that, for the UK market at least, commercial prospects do not look good.

The chapter on tidal power is largely a shortened version of the report of the Severn Barrage Committee, which concluded that up to seven gigawatts of electricity should be economically available through a barrage across the Severn Estuary in south-west England. Beyond this, there is a good summary of schemes elsewhere in the world, especially in France and Canada.

Geothermal energy from large underground steam fields is used to generate electricity economically, but this is not a practicable source for most European countries. However, there is a good introduction to world-wide use of the technology, including the use of warm water aquifers which are now being exploited in Europe for space heating. A much more widely distributed form of underground heat lies in impermeable rock, the so-called "hot dry rock" technology. In some places, for example Cornwall and Northumberland in the UK, underground rock structures have especially high temperatures so that more than 200°C can be reached at depths of 5–7 km. If one could fracture the rock at such depths and pass water over it, steam could be raised to generate electricity. There is an excellent introduction to this promising new technology, though it is made clear that it is one for the next century. Finally, the book deals with various ways to raise electricity from solar energy including photovoltaic

conversion, satellite stations and solar focusing of the Sun's heat.

Alternative Energy Sources is a well-structured compendium of recent work on the renewables, with especial reference to the UK programme. It is rather uncritical and contains no new insights. Nevertheless, the book represents a useful introduction to the subject, though some scientific education will be required to understand all of the explanations. □

F.J.P. Clarke is Director of Management Studies at the United Kingdom Atomic Energy Authority. From 1977 to 1981 he was Chairman of the UK national committees on wind, wave and solar energies.

Alluvial puzzles

Alayne Street-Perrott

A Land Between Two Niles: Quaternary Geology and Biology of the Central Sudan.

Edited by Martin A.J. Williams and D.A. Adamson.

A.A. Balkema, Rotterdam: 1983. Pp.256. Dfl.65, £14.55, \$25.

LEONARDO da Vinci speculated that the Nile floods originated in the Mountains of the Moon. Despite all the detailed work carried out subsequently on the alluvial sequence of the Nile, its Late Quaternary history is still surrounded by controversy. On the one hand, palaeoecologists working in the Nile headwaters insist that the last glacial maximum was cool and dry; on the other, painstaking stratigraphic studies in Nubia and Egypt seem to lead inescapably to the conclusion that the Late Pleistocene Nile was more vigorous and competent than today.

A Land Between Two Niles attempts to resolve these contradictions. It is an interdisciplinary study of the Gezira, which occupies a key position at the confluence of the Blue and White Niles. The Gezira is a complex alluvial fan, consisting mainly of sediments brought down by the Blue Nile from the Ethiopian Highlands. These interfinger with dune sands and with the deposits of the White Nile.

The book begins with brief surveys of the problems of irrigation agriculture in semi-arid climates, and of Quaternary environments in Northern Africa. A reconstruction of Late Quaternary vegetation changes in the Sudan is followed by six chapters dealing with the geology, hydrology, landforms, vegetation, soils and stratigraphy of the Gezira. The book concludes with a conceptual model of the fluvial dynamics of the Nile since 20,000 yr BP. At present, the Blue Nile contributes 68% of the flood discharge of the Main Nile and 72% of its sediment load. In contrast, the White Nile, which rises in the lakes and swamps of Equatorial Africa, serves mainly to maintain the low-season

flow. An important conclusion of this book is that the water and sediment discharge of the Blue Nile have been the overriding influence on the behaviour of the Main Nile throughout the late Quaternary. By taking sediment production as well as climatic change into account, the authors explain many of the puzzling features of the alluvial record.

The Gezira is a region of great economic importance, since it accounts for 65% of the export revenue of the Sudan and around a fifth of the world's production of long-staple cotton. The authors argue that future development must be based on an understanding of the extent to which the alluvial soils and groundwater reserves are out of phase with present conditions. In this sense, "the past is . . . a very real key to the present".

Not surprisingly in a work of this kind, there are occasional disagreements between contributors. More irritating to the reader are the inconsistencies between some of the maps and diagrams, which are made worse by poor reproduction in some cases. Because of the general spatial and systematic organization of the book, it



alternates between the present and the Late Pleistocene in a way which leaves the reader slightly queasy.

Nonetheless this volume is a succinct and useful summary of existing information on the past and present landscape of the Gezira. It forms a valuable companion to the earlier work *The Sahara and the Nile* (edited by M.A.J. Williams and H. Faure, and published by Balkema in 1980), and issues a clear warning to all those Quaternary scientists who would like to infer palaeoclimate from the sedimentary record of large river systems. □

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Mammoths and man

Andrew Hill

Paleoecology of Beringia.

Edited by David M. Hopkins *et al.*

Academic Press: 1983. Pp. 489. \$37, £24.60.

BERINGIA is a vast tract of land and sea near the top of the globe. The region links Siberia and Alaska, and forms the corridor along which humans, in the manner of Moses, originally reached the New World from the Old when the seas were parted. For the anthropocentrically-minded the nature and timing of this immigration is the area's principal source of fascination, but there are equally interesting problems of more general environmental and palaeoecological import. These centre on an apparent anomaly — climatic and palaeobotanical information suggest that primary productivity was unable to support the large herds of mammoth, bison, horse, reindeer and other ungulates known to have lived there.

Paleoecology of Beringia comes from one of the late, lamented Burg Wartenstein conferences of the Wenner Gren Foundation, and reflects the diversity and range that characterized many of these valuable events. It is almost mandatory these days to say of your proceedings' volume that it is 'more than a conference volume', but here it really appears to true. The book is not just a collection of disparate items assembled before a meeting, for several of the papers, and much of the material in others, are the product of fresh ideas and insights that were provoked by the conference itself. All of the contributions have been brought up to date to include work carried out subsequently, and often refer to pertinent data still in press.

Twenty-four chapters are grouped into a number of sections dealing with palaeogeography and geology, palaeovegetation, the steppe-tundra concept, palaeoclimates, the mammals and the problem with primary productivity, and man in Beringia with his possible role in mammal extinctions. Each section is drawn together by a short introduction, and the main points of the book are summarized in a long concluding chapter written by the editors.

Among the contributors are Russian workers in eastern Beringia as well as their American counterparts; another useful feature is the inclusion of reports by specialists previously unconnected with the area, such as those involved in climatic modelling, and in the study of modern elephants and the people who hunt them.

Many of the problems remain. On a conservative estimate, people appeared in Beringia about 11,500 years ago. More extreme assessments, relying upon variously acceptable data from modified animal bones, range back as far as 125,000

years. The degree to which human beings were instruments in the dissolution of the ecosystem is still debated. A number of disjunct distributions of organisms are intriguing, and the productivity paradox is still not entirely explained.

The book is a valuable and current summary of what is known about this important region in the later Pleistocene. It is interesting that the productivity paradox has necessitated an abandoning of simple modern analogies, and has led to a move away from naïve contemporary compari-

sons to something more subtle and complex. Possibly we are dealing here with something quite different from any ecosystem found today. In this, and other ways, *Paleoecology of Beringia* is methodologically instructive for ecologists who work on times even more remote, and on mammals other than mammoths and man. □

Andrew Hill is a Research Fellow at Harvard University.

Carrying iron

Takashi Yonetani

Iron Porphyrins, Parts 1 and 2.

Edited by A.B.P. Lever and Harry B.

Gray.

Addison-Wesley: 1983. Pp. 540. Part 1 £29, \$38.95; Part 2 £28.45, \$37.95.

THE AMAZING diversity of biochemical reactions catalysed by haemoproteins is well matched by the variety of the valency and spin states and redox potentials exhibited by their prosthetic group, iron porphyrins. Thus, iron porphyrins are an ideal probe through which to gain insight into the structure-function relationship of haemoproteins. These two books, together comprising the first volume of a new series, *Physical Bioinorganic Chemistry*, provide a succinct overview of modern physical methods that are being applied to the study of iron porphyrins.

Part I starts with a review by Loew of how various approaches in theoretical chemistry can be applied to correlate spectroscopically-observable properties of iron porphyrins with their molecular properties. Scheidt and Gouterman then describe high-resolution molecular structures of iron porphyrins, and present semi-quantitative, theoretically predictable correlations between the valency and spin states of the metal ion, the nature of axial ligands, and the geometric structure of the molecule. In the following chapter, an incisive account of the analysis of the electronic spectra of haemoproteins by polarized single-crystal spectrophotometry, Makinen and Churg give a vivid demonstration of the importance of the interplay between theory and experiment in interpreting the complex electronic spectra of iron porphyrins and haemoproteins. Finally, Goff describes some well-selected nuclear magnetic resonance studies, especially paramagnetically-shifted nuclear magnetic resonance, to demonstrate the analytical capabilities of the technique in investigating the electronic, geometric and dynamic properties of iron porphyrins in solution.

Part II opens with an article by Mitra on magnetic susceptibility; highly orientated towards physics and chemistry, this

introduces theoretical and experimental approaches to iron porphyrins. Palmer covers theoretical and experimental aspects of electron paramagnetic resonance of haemoproteins, while Spiro's account of resonance Raman spectroscopy of metalloporphyrins and haemoproteins provides a good introduction to this relatively new spectroscopic technique which has emerged to provide precise data on the geometric, electronic and dynamic properties of metalloporphyrins in solution. Concluding the second volume is an article by Kadish on the electrochemistry of iron porphyrins; here the author gives exclusive attention to experimental aspects of redox-potential measurements of iron porphyrins in non-aqueous media.

Some contributors have chosen to limit themselves to either iron porphyrins or haemoproteins, even though a number of advances have been made in other areas. Nonetheless all the contributions are based on well-established theoretical and experimental results, so that together these two volumes will serve as a valuable reference work. The reader will require a basic knowledge of physical chemistry to fully appreciate many of the techniques discussed, but clear descriptions, cogent explanations and well-chosen illustrations will make the books useful to the non-specialist wanting a concise overview of these modern methods. In this day of specialization, however, the comprehensive coverage will perhaps be valued most by experts wishing to acquaint themselves with recent progress in techniques allied to their own. □

Takashi Yonetani is Professor of Biochemistry and Biophysics at the University of Pennsylvania, Philadelphia.

A fresh analysis

The first volume of a third edition of *Methods in Enzymatic Analysis*, edited by H. Bergmeyer and published by Verlag Chemie, Weinheim, is now available.

The first single-volume edition, published in 1963, was followed by a second edition of four volumes in 1974. To keep pace, ten volumes are planned for this edition. The price is DM 230 for a single volume but DM170 for those who subscribe to the complete edition.

Cutaneous matter

Sam Shuster

Skin.

By P.F. Millington and R. Wilkinson.
Cambridge University Press: 1983.
Pp.224. £35, \$69.50.

DERMATOLOGY is still practised as a visual art. Why not, if the eye is still the best instrument available. Is it the clinician's problem that visual classification is a cliché no longer capable of advancing the subject? Of course the clinician's eye is short-sighted, but that myopia won't be corrected until clinical science is clearly shown to have inescapable consequences for the day-to-day business of clinical practice.

I therefore welcome the new interest in skin biology of which this book is a reflection. But it was not just the acuteness of need and intensity of anticipation that made the inadequacies of this book so stark: it fails in its own right, and badly.

I never judge a book by the word-dust on its jacket but the clap-trap of "interaction and dependence on cell turnover, nutrition, control, mechanisms and disease" is the only reference to purpose. The preface opts for an "overview" by "selecting" to

"illustrate"; but for whom? The book is too slight for the expert and too unbalanced for the novice, both faults being unmitigated by interest or novelty. The attempt to inform about skin structure and function is dated, inaccurate and confusing — the incredible attribution of cutaneous pain to histamine; the primitive account of epidermal kinetics; the confusing juxtaposition of historical with contemporary views on the Langerhan cell; the embarrassing references to discredited work on sebaceous glands; the recommendation that "primary stimulation [of sweat glands] may be achieved by drugs such as 0.5–1.0mg acetyl salicylic acid followed by copious quantities of water or tea". My list of comic quotes is not much shorter than the text itself.

These serious deficiencies go far beyond the book itself and raise a number of questions about the sorry state of biomedical writing and publishing. Inevitably texts get written by people better able to make science than write about it. It does

not help that we are encouraged, if not importuned, by text-hungry publishers who make little or no attempt to be sure we know our subject or can write about it, and who accept almost anything on the nod. This uncritical and unselective role of biomedical publishers is the most important reason for the existence of so many poor books.

Ultimately it is the technical publishers' responsibility to maintain quality just as their colleagues do with works of fiction. To do this they must increase their technical understanding to match their literary and publishing skills, or take more advice. Until biomedical publishers make greater efforts to improve the appalling standards of their craft, more books will fail than succeed and our libraries will continue to be choked with expensive irrelevancies. □

Sam Shuster is Professor of Dermatology at the University of Newcastle upon Tyne.

At the interface

Frank H. Stillinger

Molecular Theory of Capillarity.

By J.S. Rowlinson and B. Widom.
Oxford University Press: 1983. Pp.327.
£30, \$59.

WHENEVER substances can undergo first-order phase change, coexistence of distinct macroscopic phases becomes possible with a separating interface in evidence. This monograph is devoted to selected aspects of the theory for such interfaces, specifically those between fluids.

This subject is now large, though it is pedagogically still incomplete. As a result, the authors wisely have limited themselves to a relatively unified portion of the field that closely fits their own recent research interests. Thus little emphasis is placed on quantitative measurements (e.g. surface tension) of real substances; rather the goal has been to clarify general theoretical principles and to explain interfacial phenomena qualitatively in terms of rigorous thermodynamics and statistical mechanics. No kinetic phenomena are considered, only static behaviour.

The density distribution of matter through an interface and the associated surface tension are manifestations of intermolecular forces, a fact appreciated long ago by Laplace. But even today these forces are known with precision only for the simplest of substances such as the noble gases. For that reason, Professors Rowlinson and Widom have restricted their discussion principally to those simple substances and their liquid mixtures, as well as to a few equally simple theoretical models (e.g. the Ising model and the

"penetrable sphere" model) in which interactions are given by fiat.

The book thus has a mildly reductionist flavour: unless it is possible to comprehend these elementary examples it is hopeless to undertake more complicated cases. Unfortunately, this excludes discussion of many fascinating and important phenomena such as the preferred orientation of polar molecules at interfaces, the structure of liquid-crystal and molten metal surfaces, and the nature of amphiphile aggregates in solution (micelles, microemulsions, membranes).

The authors have provided a solid historical background for their subject, a useful guide to recent research papers, and a tour through basic concepts and theoretical techniques of both phenomenological and molecular character. In addition to summarizing arguments and presenting useful formulae, they indicate points of weakness in the field. One concerns reconciliation of formulae for position of the so-called "surface of tension". Another and more serious issue involves definition of an inherent density profile for an interface that is free of the influence of thermally-driven capillary waves which cause the interface width to diverge in the zero-gravity limit.

Because of rapid progress in interface theory, the case for a revision of the present text will eventually become compelling. In particular, greater attention will have to be given to renormalization group techniques (briefly sketched in this edition) and to computer simulation (now one very short chapter). In the meantime anyone serious about research on interfacial properties should own, or at least have direct access to, this monograph. □

Frank H. Stillinger is at Bell Laboratories, Murray Hill, New Jersey.

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American Chemical Society Meeting

The products described in this week's feature include those being presented at the American Chemical Society Meeting, to be held in Washington DC, USA, 28 August–2 September 1983.

Spectrometry

● Two liquid-nitrogen-cooled mercury cadmium telluride (MCT) detectors for the model 1500 Fourier transform IR spectrophotometer have been introduced by Perkin Elmer. Two MCT detector models are available: the wideband version, which can be used over almost the full range of the model 1500 ($4,400\text{--}450\text{ cm}^{-1}$), and the narrowband version, used over the range $4,000$ to 740 cm^{-1} . The new detectors can be used where sample transmission is very low, such as in the use of carbon-filled polymer films, and diffuse and specular reflectance sampling.

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● Two new mass spectrometer systems have been released by Finnigan Mat — the ITD 700 and the 271/45. The ITD 700 is a detector system for gas chromatography which operates on the principal of "ion trapping", whereby ions are generated and separated. The 271/45 is a mass spectrometer system designed to measure gas mixtures.

Circle No. 101 on Reader Service Card.

● Finnigan Mat have introduced a gas chromatography/mass spectrometry system, called the model 5100, for research applications in analytical chemistry. The system includes the Incos data system and uses the SuperIncos software. This allows, for example, simple descriptors to be created to sequence the ionizer filament to scan ranges, and to detect positive and negative ions during an experiment. The system has a 35-Mbyte Winchester disk drive, a new video terminal and a detachable keyboard which can be moved up to two feet from the terminal. The analytical capabilities of the 5100 include chemical, electron, and discharge ionization, along with pulsed positive ion/negative ion chemical ionization (PPINICI). The inlet devices available include a solids-probe, a direct-insertion probe (DEP), and a 60-vial autosampler.

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● Shimadzu have released a new double-beam, microprocessor-controlled spectrophotometer, the UV-250. The unit is based on the UV-240 and features the same graphic printer/plotter and optional modules; but also includes a double-monochromator. This has both a plane and a concave holographic grating on an aberration-corrected Czerny-Turner mounting. The double monochromator gives very little stray light (less than 0.001%), and produces photometric linearity up to 5.0 Abs.

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● Varian Associates has introduced a new NMR data system for its XL series of 200, 300 and 400 MHz superconducting Fourier transform NMR spectrometers. The new system, called Advance, is based on a series of independent computers which are linked by a 32-bit bus structure. Task management is by a disk-based Pascal operating system. With its optional array processor, Advance can perform 8,000 floating-point Fourier transforms in 500 ms. The system has a 16-colour graphics display, and 464 kbytes of memory, which is expandable to over 16 Mbytes. The two major computer systems, the host system and the acquisition system, are based on the Motorola 68000 microprocessors.

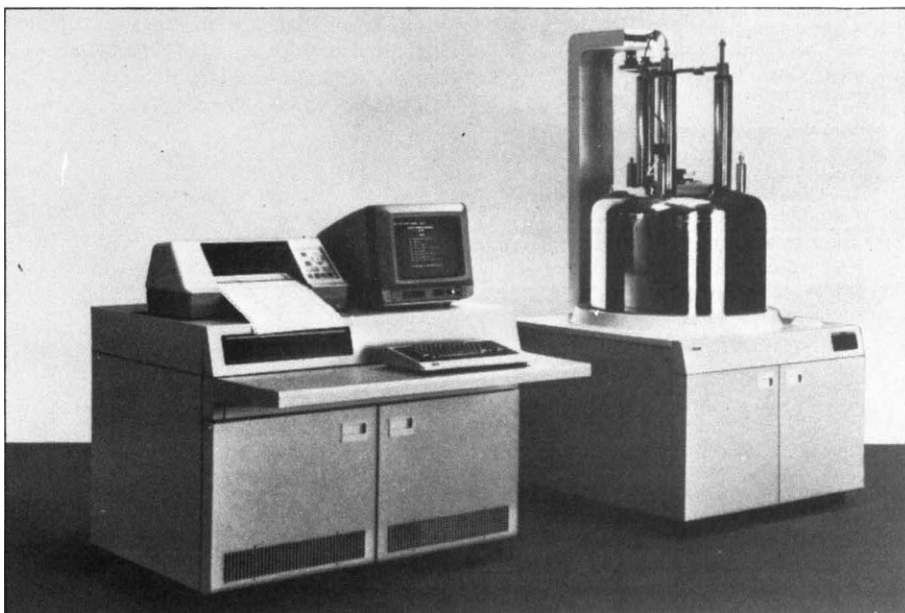
Circle No. 104 on Reader Service Card.

● VG Analytical has extended its range of mass spectrometers with the introduction of the VG ZAB-E. The new instrument has a mass range of 10,000 AMU at 10 kV accelerating voltage (12,500 AMU at 8 kV). It can also be linked to the VG11-250 data system which itself has a mass range of 16,000 AMU. The accelerating voltage and extended geometry of the VG ZAB-E increase its sensitivity, and specifications for the resolution and the maximum scan rate are 100,000 and 0.15 seconds per decade respectively.

Circle No. 105 on Reader Service Card.

● The new NMR system from Nicolet is designed primarily for routine proton and carbon NMR. The QE-300 is based on a superconducting 300 MHz magnet and has synthesizer-based frequency control, homonuclear and heteronuclear proton decoupling, a high-speed pulse programmer, a switchable proton-carbon probe, and menu-driven software. The system also includes a colour raster display, an eight-pen plotter, and dual 8-inch floppy disk drives. All spectrometer functions in the QE-300 are under computer control with automatic ^1H to ^{13}C conversion, spin control, magnet shimming, frequency locking, data acquisition, spectral phasing, plotting, and peak integration.

Circle No. 106 on Reader Service Card.



The new QE-300 NMR system from Nicolet

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● A series of new features has been announced by Philips for the modular PV 8350 vacuum spectrometer system. The spectrometer optics now include analytical lines at longer wavelengths. With the standard 2,160 lines per mm grating, for example, the lines Na 588.9 nm and Li 610.3 nm can now be measured in the first order. For the analysis of metals, a new 50 Hz source (PV 8560) combines monoalternance excitation with computer-controlled high-energy preburn, for installations where the speed of the Philips PV 8350 500 Hz source is not required. For PV 8350 spectrometer systems which include an inductively-coupled plasma source unit, other accessories are now available. These include a choice of automatic systems for handling batches of solutions or measuring by multi-element standard additions.

Circle No. 107 on Reader Service Card.

● A new spectrophotometer from Oriel Scientific, the Turner model 340SQ, is now available with a square cuvette holder. The instrument has a wavelength range of 330 to 1,000 nm which can be extended to 200 nm with a UV accessory.

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General laboratory equipment

● Nucleon, from National Diagnostics, is a solution for removing radioactivity from laboratory surfaces, glassware and equipment. It is biodegradable and mild to the skin when used as directed. It is supplied in quart containers with a spray head, or in gallon quantities.

Circle No. 109 on Reader Service Card.

● A new family of badges for monitoring organic vapour has been introduced by SKC. The badges are offered with any one of five sorbents. Each badge has a back-up section to assure total sample collection and the sorbent is retained in a replaceable capsule. Reusable holders can accommodate one or more sorbent capsules for single, double or multi-sorbent sampling. After the sample has been obtained it is analysed in the laboratory.

Circle No. 110 on Reader Service Card.

● FTS Systems has introduced a new line of low-temperature mechanically-refrigerated traps for collecting condensable vapours in vacuum systems. The traps are available in three configurations, with condenser temperatures of -54°C, -84°C and -130°C.

Circle No. 111 on Reader Service Card.

● A new range of calibration masses for electronic balances is now available from Astell Hearson. The brass masses are supplied either lacquer-coated or chromium-plated. They are supplied either in sets with a polypropylene storage case or as individual masses.

Circle No.112 on Reader Service Card.

● UV eye and face protection units are now being produced by Blak-Ray. These units ensure that transmission of UV light at 254, 302 and 365 nm is virtually eliminated. The full range of protective units from Blak-Ray includes a UVC-803 tip-up visor face shield, UVC-503 goggles and UVC-303 spectacles.

Circle No. 113 on Reader Service Card.



Protection units from Blak-Ray

● A general-purpose laboratory pipettor, diluter or dispenser with an accuracy and a precision of ± 1% at total syringe volume is now being offered by Dynatech Laboratories. This syringe, the DSD-100, is the simplest model in a new range of digital syringe diluters. The DSD-100 is a portable, desk-mounted unit which uses thumbwheels to control both total and sample volume. It has a microprocessor-controlled stepping-motor drive system with a separate, synchronous motor to power the valves. The syringe is made of glass and Teflon.

Circle No.114 on Reader Service Card.

● Astell Hearson have now produced a new range of port-o-gram balances. Two weighing ranges are available: 300×0.1 g and 3,000×1 g. All models have a large, unobstructed, stainless steel platform, and a low-battery indicator warns when batteries need replacement.

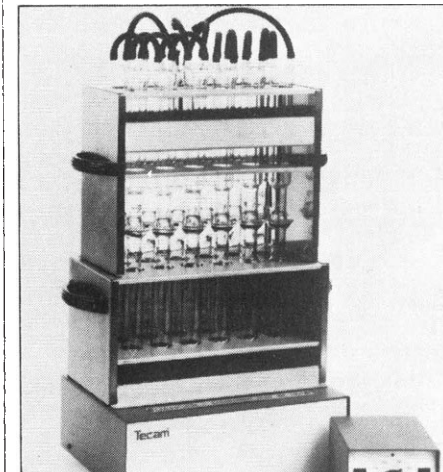
Circle No.115 on Reader Service Card.

● V.A. Howe now produce a range of digital temperature-measuring and control instruments which cover the range -220 to 1,370°C. The instruments may be used with either diode, platinum-resistance or thermocouple sensors.

Circle No.116 on Reader Service Card.

● A compact block heater system for use in the determination of chemical oxygen demand (COD) is being produced by Techne. The COD test for determining the concentration of dissolved oxygen involves heating the sample under reflux to a temperature of 150°C. The new dry block allows uniform heating of samples, and gives higher sample throughput by virtue of its reaction time. In addition, the Techne dry block units have interchangeable insert blocks so that one heater unit can be used for a variety of sizes of tube. The new system can analyse up to twelve 26-mm tubes simultaneously.

Circle No. 117 on Reader Service Card.



Techne's block heater system

● The refrigerated bench centrifuge from Hettich Zentrifugen, the Mikro Rapid/K, is designed for use with Eppendorf, Beckman or Becton Dickinson microtubes. The centrifuge can be used to carry out haematocrit counts if a centrifuge plate is attached, and has angle heads of 12×10 ml, 28×1.5 ml and 22×1.5 ml. It also contains hermetic refrigerating equipment. A thermostat controls the temperature between 0 and 50°C , and the working temperature, which depends on the maximum speed of the rotors, ranges from 0 to 5°C . The speed is automatically controlled through a thyristor, and the instrument can be electronically braked after a preselected time.

Circle No. 118 on Reader Service Card.

● Labconco now offers the Fume Adsorber-2, a double-width, enclosed work-station. The work-station has two $1\frac{1}{2}$ -inch-deep carbon filters, extending across the enclosure, each of which can adsorb odours and fumes of up to one third its own weight.

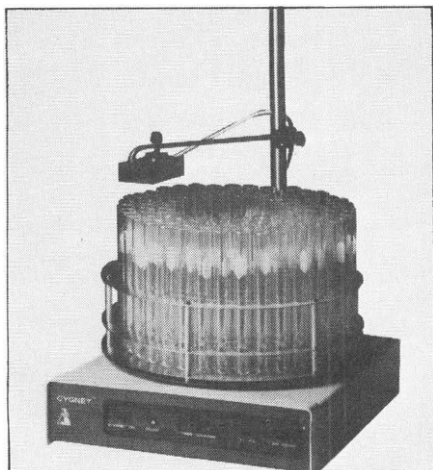
Circle No. 119 on Reader Service Card.

● ChemLab Instruments have added a new chloride meter to their range of products. The model CCM.I provides a digital readout of the chloride concentration in biological samples such as serum, plasma, sweat, cerebrospinal fluid and urine. Up to 20 samples can be measured in one aliquot of acid buffer solution. An integral warning light indicates when the chloride concentration of the solution has reached the 2,000 mmol per litre level and needs replacing. The model CCM.I is supplied with electrodes, detectors, stirring bars and an initial supply of standards, reagents, electrode polish and beakers.

Circle No. 120 on Reader Service Card.

● Isco's new "Cygnets" fraction collector can count fractions by time, volume or drop. It has a fast indexing system, which can be used for HPLC. In addition it can hold 25 ml tubes, which means that it can be used for low pressure chromatography.

Circle No. 121 on Reader Service Card.



The Cygnets fraction collector

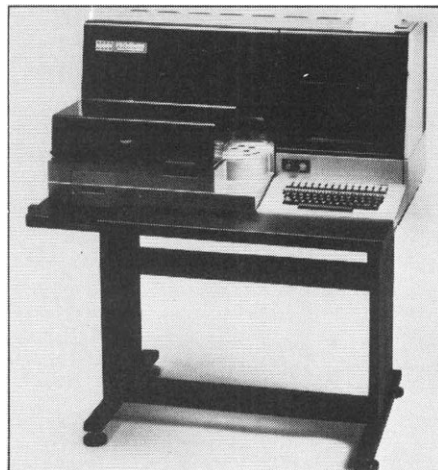
Analysers

● A new selective clinical analyser, called Progress, has been developed by the Kone Corporation. The Kone Progress is a selective discrete analyser which gives random access to samples during analysis. The system is completely automatic and will perform dilution and re-analysis of results which are out of range. The tests that can be carried out include endpoint, bichromatic and kinetic assays with 12-point linearity control, together with non-linear calibration for immunoassays. The tests can be defined by the user. The Progress can maintain a continuous throughput of 400 tests per hour for one day. While individual or panel selection of up to 30 tests is available for every sample, the Progress microcomputer constantly selects the optimal order of analysis to obtain maximum throughput. The system uses microcuvettes, so that typical reagent volumes in the Kone Progress are below $100\ \mu\text{l}$. The Progress analyser incorporates a data-management system which provides collated patient reports, sorted by ward, and quality control facilities. Interfaces are provided for a main laboratory computer (bidirectional link), a slave VDU, and a printer and optical reader. The system requires mains power and is provided with battery back-up facilities to cope with mains power failure.

Circle No. 122 on Reader Service Card.

● An interactive, microprocessor-based electrochemical analyser has been introduced by BAS. The system has a repertoire of 20 techniques, including that of square wave voltammetry. Adjustment of experimental parameters, as well as signal processing, is controlled using the keyboard, and data may be presented on both the video screen and the digital plotter. The new BAS-100 electrochemical analyser makes it possible to perform most electroanalytical techniques with one machine where several were previously required.

Circle No. 123 on Reader Service Card.



The Kone Progress

● Nelson Analytical has released a new software package dedicated to amino acid analyser data reduction. Data from any commercial dual-column or dual-wavelength amino acid analyser can be processed by the Nelson Analytical model 4416 data system with this software program. This new software package allows data on the sample or the patient to be stored along with the amino acid analysis data. The model 4416 can acquire, digitize, and store the data from the amino acid analyser at two different channels or wavelengths; it can compute amino acid concentrations, and automatically determine the correlation of the sample with amino acid compositions of samples from different age groups. The system is also able to eliminate false positives in the data through automatic comparison of the ratios of the two wavelengths so that only genuine amino acids are treated in the calculation. Amino acids are specifically listed by name. The model 4416 consists of the 16-bit HP series 200 model 16 desktop computer with dual microfloppy disk drives, a printer/plotter, an instrument interface, and chromatography software. The model 4416 is also capable of standard chromatography data reduction to generate area percent, internal and external standard, and normalized area percentage calculations. Raw data is stored in the system so that it is possible to carry out post-run operations, compare stored data, reintegrate the data with new integration parameters, and recalculate the data with different baseline settings.

Circle No. 124 on Reader Service Card.

● A gas analyser that allows all gaseous elements with molecules of atomic weights in the range between 1 and 100 AMU to be detected has been produced by Leybold Heraeus. Called the Quadrupole Q 100, the analyser is a quadrupole mass spectrometer which is designed for routine process monitoring and control. The analyser can also be used to monitor heavier molecules, such as linear hydrocarbon chains, since they can be decomposed into lighter fractions by high-energy electron bombardment. Hence the Q 100 is suitable for the analysis of contaminants in vacuum processes. In vacuum processes where the working pressure is too high for direct gas analysis by a quadrupole mass spectrometer, a pressure converter system can be used with the Q 100 to reduce the process pressure to the typical operating pressure of the system without influencing the original composition of the gas mixture under analysis.

Circle No. 125 on Reader Service Card.

● World Precision Instruments (WPI) has introduced a new microanalytical instrument, the Picapnotherm, model GV-1. The instrument analyses carbon dioxide in fluid samples by differential temperature reaction.

Circle No. 126 on Reader Service Card.

Chromatography

● The Desaphor VA electrophoresis system has been developed by Desaga for use in polyacrylamide gel electrophoresis. The gel width is uniform at 230 mm with separation distances of 110, 150, 200 and 300 mm. Separation cells with both plastic and glass spacers are available for all separation lengths. There are separation cells of 110 and 220 mm in length for preparative polyacrylamide gel electrophoresis in 11 cm-thick gels. A built-in buffer circulation unit coupled with a heat exchanger makes it possible to carry out electrophoresis up to 250 W.

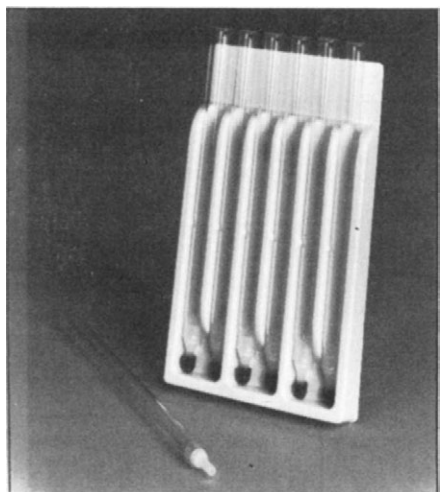
Circle No. 127 on Reader Service Card.

● V.A. Howe has launched a new portable dissolved oxygen meter. The meter, model 3022, reads in the range 0–100% oxygen saturation and oxygen content, and 0–19.99 p.p.m. oxygen. It also has a –40 to 150°C temperature measurement facility. In the oxygen modes, resolution is 0.1% or 0.01 p.p.m. and the system is capable of handling measurement at the sub 1 p.p.m. level. The meter has a revised probe permitting fast, reliable readings to be made with auto-temperature compensation built into the probe. A large potassium chloride reservoir reduces membrane dessication and the probe has a removable, protective 'boot' fitted over the membrane to shield it, and is supplied with a rubber 'boot' to keep the membrane wet during transport and storage. Two sizes of cases are also available, one for the meter and accessories and one to carry a portable battery stirrer as well.

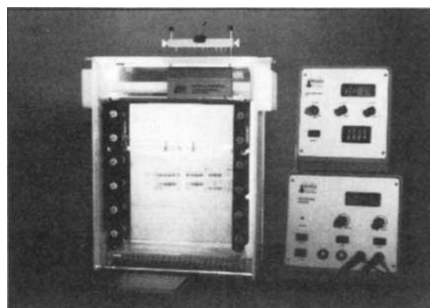
Circle No. 128 on Reader Service Card.

● A new disposable glass chromatography column is available from Kontes. Supplied in packages of 48, each unit consists of a 150×8 mm (inside diameter) borosilicate glass column, a 30–50 µm porous polyethylene disc, and a moulded polypropylene lower luer fitting.

Circle No. 129 on Reader Service Card.



The chromatography column from Kontes



The Desaphor VA electrophoresis system



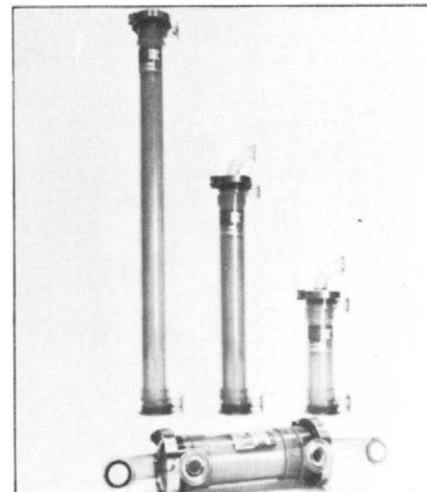
Eldex Laboratories' pump monitor

● A new pump monitor designed for pressures up to 6,000 p.s.i. is now available from Eldex Laboratories. The unit features an integral pulse damper to smooth solvent delivery and to reduce baseline noise, thus increasing detector sensitivity. An LCD readout provides a display of system pressure in either p.s.i. or bars. Slide adjustments on the front panel fix high and low pressure limits. In the event of accidental system leakage or clogging, the power supply to the pump is automatically cut when either pressure limit is reached.

Circle No. 130 on Reader Service Card.

● Romicon has introduced a new 'short-short' model to its line of hollow-fiber ultrafiltration cartridges. It was designed to enable the processing of materials with high solids concentrations and to cope with products exhibiting viscosity handling problems. The new unit, which is half the length of standard cartridges, operates at higher cross-flow velocities and higher operating pressures than standard lengths. This enables it to handle materials which normally show unacceptably low fluxes when processed with currently-available membrane systems.

Circle No. 131 on Reader Service Card.



Romicon's 'short-short' model



The Sartophor electrophoresis system

● The Sartophor system for electrophoresis is for use in microanalytical and preparative electrophoresis and isoelectric focusing methods. A complement of accessories for cellulose acetate and gel electrophoresis (including a heat transfer cooling system) and reagents for clinical diagnostic testing and isoelectric focusing are also available.

Circle No. 132 on Reader Service Card.

● The model 201 fraction collector software from Anachem includes a new peak-detection algorithm combining both slope and level detection. This is designed to aid peak collecting during HPLC gradient runs with drifting baselines. The system has a floating decimal point and incorporates "free-vessel" programming to allow collections into vessels of virtually any size and shape anywhere in the indexing head pattern. A running program can be "held" and its parameters changed to suit new requirements.

Circle No. 133 on Reader Service Card.

● Kratos Analytical Instruments has introduced new post-column derivatization systems for HPLC. These new systems provide column temperature control and post column derivatization reagent flow control (for one or two reagents), whilst controlling post column reaction temperature and detecting sensitive fluorescence or absorbance. Recorders and integrators are optional.

Circle No. 134 on Reader Service Card.

● A new high performance liquid chromatograph has been produced by Waters Associates. The QA-1 analyser is an automated HPLC system designed to perform repetitive analyses unattended. A new accessory is available that allows the system parameters to be set and locked in. This can be reset at any time. The QA-1 analysers can now accommodate up to 96 samples per loading. Systems are available for operation at pressures up to 2,000 or 4,000 p.s.i.

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